Chronic Interactions Between Carotid Baroreceptors and Chemoreceptors in Obesity Hypertension

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See Editorial Commentary, pp 24–26

Abstract—Carotid bodies play a critical role in protecting against hypoxemia, and their activation increases sympathetic activity, arterial pressure, and ventilation, responses opposed by acute stimulation of the baroreflex. Although chemoreceptor hypersensitivity is associated with sympathetically mediated hypertension, the mechanisms involved and their significance in the pathogenesis of hypertension remain unclear. We investigated the chronic interactions of these reflexes in dogs with sympathetically mediated, obesity-induced hypertension based on the hypothesis that hypoxemia and tonic activation of carotid chemoreceptors may be associated with obesity. After 5 weeks on a high-fat diet, the animals experienced a 35% to 40% weight gain and increases in arterial pressure from 106±3 to 123±3 mm Hg and respiratory rate from 8±1 to 12±1 breaths/min along with hypoxemia (arterial partial pressure of oxygen=81±3 mm Hg) but eucapnia. During 7 days of carotid baroreflex activation by electric stimulation of the carotid sinus, tachypnea was attenuated, and hypertension was abolished before these variables returned to prestimulation values during a recovery period. After subsequent denervation of the carotid sinus region, respiratory rate decreased transiently in association with further sustained reductions in arterial partial pressure of oxygen (to 65±2 mm Hg) and substantial hypercapnia. Moreover, the severity of hypertension was attenuated from 125±2 to 116±3 mm Hg (45%–50% reduction). These findings suggest that hypoxemia may account for sustained stimulation of peripheral chemoreceptors in obesity and that this activation leads to compensatory increases in ventilation and central sympathetic outflow that contributes to neurogenically mediated hypertension. Furthermore, the excitatory effects of chemoreceptor hyperactivity are abolished by chronic activation of the carotid baroreflex. (Hypertension. 2016;68:227-235. DOI: 10.1161/HYPERTENSIONAHA.116.07232.)

Key Words: baroreflex • blood pressure • carotid bodies • hypertension • obesity • sympathetic nervous system

Stimulation of peripheral chemoreceptors by hypoxemia plays a key role in the reflex regulation of respiration.1 In addition to raising the arterial partial pressure of oxygen (PaO₂) by increasing minute ventilation, activation of the carotid bodies also increases sympathetic outflow. Based on exaggerated increases in sympathetic activity, arterial pressure, and ventilation to chemoreceptor stimulation by hypoxia2,3 and to normalization of these variables by deactivation of chemoreceptors by hyperoxia,4–7 studies in the spontaneous hypertensive rat (SHR) and in patients with primary hypertension support the speculation that tonic increases in peripheral chemoreceptor activity may contribute to sympathetically mediated hypertension. However, the mechanisms that account for the development of carotid body hyperactivity and the potential for this in contributing to the sustained sympathetic activation that is prevalent in primary and resistant hypertension remain unclear.

Recent experimental studies and clinical trials have led to a resurgence of interest in the hypothesis that carotid bodies may drive the neurogenic component of some forms of hypertension.1,8 Chronic studies in the SHR support the hypothesis that chemoreceptor hyperactivity may contribute to sustained increases in sympathetic activity, including renal sympathetic activity (RSNA), that lead to hypertension.7,9 In these studies, denervation of the carotid bodies (CBD) by bilateral carotid sinus nerve ligation produced sustained suppression of heightened RSNA and attenuated both the development and maintenance of hypertension in the SHR. These findings are of particular interest given the clinical need for nonpharmacological approaches for the treatment of resistant hypertension.

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This, in addition to the potential translational significance of these observations in the SHR, has led to ongoing proof-of-concept clinical trials designed to evaluate the antihypertensive efficacy of unilateral carotid body resection in this patient population with a high risk for morbidity and mortality (clinicaltrials.gov identifier: NCT1745172, NCT02099851).

Activation of the arterial baroreflex inhibits sympathetic activity and lowers arterial pressure, responses opposite to those produced by stimulation of the carotid bodies. Furthermore, in work by Heistad et al.10,11 and Somers et al.,12 acute activation of the baroreflex has been shown to diminish the cardiovascular and ventilatory responses seen with stimulation of peripheral chemoreceptors. However, it remains unknown whether the baroreflex can chronically oppose the effects of peripheral chemoreceptor activation. One technique for evaluating the potential chronic role of the baroreflex in cardiovascular homeostasis uses electric stimulation of the carotid baroreflex, which provides a nonpharmacological method for chronically suppressing sympathetic activity and lowering arterial pressure. Using this technology, significant antihypertensive responses have been reported both in experimental animals and in patients with resistant hypertension.13–17

Obesity is common in resistant hypertension and likely contributes to the prevailing sympathetic activation and hypertension.13–15,18–20 However, the afferent mechanisms that account for sympathetic overactivity in obesity are incompletely defined. Because increased metabolic rate and impaired respiratory mechanics are prevalent in obesity,21–24 it remains unknown whether the baroreflex can chronically oppose the effects of peripheral chemoreceptor activation. One technique for evaluating the potential chronic role of the baroreflex in cardiovascular homeostasis uses electric stimulation of the carotid baroreflex, which provides a nonpharmacological method for chronically suppressing sympathetic activity and lowering arterial pressure. Using this technology, significant antihypertensive responses have been reported both in experimental animals and in patients with resistant hypertension.13–17

Experimental Protocol

During the control period (the days immediately preceding fat feeding) and on the last 2 days of each protocol (see below), blood samples (∼10 mL) were taken from 1 of the 2 arterial catheters. Arterial pressure was sampled continuously at 100 samples/s, 24 hours/d, using a Power Laboratory data-acquisition system (ADInstruments) and displayed and recorded on a computer for subsequent analysis.27 The daily values for mean arterial pressure and heart rate were averaged between 11:30 am and 7:30 am.

Estimation of respiratory rate was based on respiratory sinus arrhythmia (please see online-line Data Supplement for more detailed explanation). Because the heart period oscillates in synchrony with respiration, generating the phenomenon of respiratory sinus arrhythmia,28 respiratory rate was estimated by counting the number of minima in the pulse interval time series every minute (corresponding to the periodic increase in heart rate during inspirations). Using a specially designed VBA macro programming software and Excel (Microsoft, Seattle, WA), 18-hour-long segments originating from the blood pressure signal recorded continuously and sampled at 100 Hz were subjected to 3 consecutive moving window filters to extract successively local maxima (systolic blood pressure and diastolic notch, first filter) and to generate time series of systolic blood pressure (second filter) and pulse interval (time difference between 2 successive peaks) and finally respiratory rate (third filter) (Figure 1). Respiratory oscillations that extended over 2 consecutive minute intervals were counted only once and finally a 3-point moving average smoothing function was applied to the daily time series.

Summary of protocols:

1. Control (days -2 to 0)
2. Days 1 to 35, high fat, (developmental phase of obesity hypertension)
3. Days 36 to 70, reduced fat (established phase of obesity hypertension)
   - Days 36 to 40, reduced fat
   - Days 41 to 47, baroreflex activation (7 days)
   - Days 48 to 56, recovery (9 days)
   - Day 57, bilateral CBD
   - Day 70, end of study (14 days after CBD)

During the first 2 days of baroreflex activation (days 41–42), the pulse generator was programmed to deliver a continuous train of constant current impulses using the following parameters: 3 to 6 mA, 30 Hz, and 0.5 ms pulse duration. The intensity of activation was selected by adjusting the current to target a reduction in arterial pressure from hypertensive to control levels. To achieve this goal, small adjustments in current were needed during the first 48 hours, but no changes in the intensity of activation were made after the first 48 hours of stimulation.

Analytical Methods

Arterial blood gas samples were analyzed immediately on a blood-gas analyzer (ABL80 FLEX; Radiometer). All other blood samples were placed on ice, centrifuged, and the plasma stored at −80°C until analysis (with the exception of plasma protein concentration, which was measured before freezing). Plasma renin activity and plasma levels of aldosterone, cortisol, and insulin were measured by radioimmunoassay in the Departmental Core facility.25,26 Plasma norepinephrine (NE) concentration was measured by high-performance liquid chromatography with electrochemical detection in the laboratory of Dr David S. Goldstein.29 Plasma glucose concentration was measured with the glucose oxidation method.25,29 Standard techniques were used to measure hematocrit and the plasma concentrations of sodium, potassium, and protein.25,29

Breathing Rate Response to Baroreflex Activation in Nonobese Dogs

We also determined the effect of chronic baroreflex activation on respiratory rate in 6 nonobese normotensive dogs included in 1 of our recent studies.31 In this earlier study, there was a targeted and
sustained reduction in mean arterial pressure of ≈15 mmHg during baroreflex activation.

**Statistical Analyses**

Results are expressed as means±SE. One-way repeated-measures analysis of variance, followed by the Holm-Sidak test for multiple comparisons (Prism 6.05; GraphPad Software), was used to compare the following experimental periods: (1) last 3 days of established obesity preceding baroreflex activation (days 38–40) versus control (mean of days -2 to 0); (2) baroreflex activation (days 41–47) versus baseline of established obesity (mean of days 39–40); and (3) CBD (days 61–70) versus recovery after baroreflex activation (mean of days 55–56). Statistical significance was considered to be *P*<0.05.

**Results**

**Developmental Phase of Obesity Hypertension**

The hemodynamic, neurohormonal, and metabolic responses during the developmental phase of obesity were similar to those reported previously²⁵,²⁶ and are illustrated, in part, in Table 1. Further description of these changes is presented in the online-only Data Supplement. Respiratory rate increased from 8±1 to 12±1 breaths/min with weight gain, a new finding not previously reported in this model of obesity hypertension.

**Established Phase of Obesity Hypertension**

During the transition from high to reduced fat intake (days 36–40), there were no significant changes in sodium balance, arterial pressure, respiratory rate, or in any other measured variable including those listed in Table 1.

**Responses to Baroreflex Activation**

As illustrated in Figures 2 and 3, baroreflex activation completely abolished the hypertension and greatly diminished the tachycardia and tachypnea associated with weight gain. Further, the lowering of arterial pressure with baroreflex activation occurred concurrently with significant reductions in both plasma NE concentration and plasma renin activity (Table 1), consistent with previous observations in obese dogs.²⁵,²⁶ As reported previously,²⁵,²⁶ there were no significant changes in body weight or sodium balance during baroreflex activation when compared with prestimulation values (day 40). Other than the reductions in plasma renin activity and plasma NE concentration, there were no significant changes in the plasma levels of any measured hormones, glucose, electrolytes or in hematocrit. After terminating baroreflex activation, all of the above measures returned to values that were not significantly different from the prestimulation levels on day 40.

**Table 1. Neurohormonal and Metabolic Responses to Baroreflex Activation and Carotid Body Denervation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>PRA, ng ANG/mL/h</th>
<th>P&lt;sub&gt;ald&lt;/sub&gt;, mg/dL</th>
<th>P&lt;sub&gt; cort&lt;/sub&gt;, μg/dL</th>
<th>P&lt;sub&gt;&lt;sup&gt;r&lt;/sup&gt;glu&lt;/sub&gt;, mg/dL</th>
<th>P&lt;sub&gt;&lt;sup&gt;r&lt;/sup&gt;ins&lt;/sub&gt;, μU/mL</th>
<th>P&lt;sub&gt;&lt;sup&gt;r&lt;/sup&gt;nep&lt;/sub&gt;, pg/mL</th>
<th>BW, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0±0.1</td>
<td>5.5±0.8</td>
<td>2.0±0.4</td>
<td>121±36</td>
<td>8.2±0.8</td>
<td>108±5</td>
<td>24.7±0.3</td>
</tr>
<tr>
<td>High fat</td>
<td>2.6±0.6</td>
<td>7.4±1.5</td>
<td>2.5±0.5</td>
<td>132±23</td>
<td>20.1±2.5*</td>
<td>93±5</td>
<td>33.8±1.2</td>
</tr>
<tr>
<td>Reduced fat</td>
<td>1.7±0.5</td>
<td>5.6±0.5</td>
<td>2.9±0.8</td>
<td>139±23</td>
<td>17.2±2.3*</td>
<td>106±3</td>
<td>33.7±1.4</td>
</tr>
<tr>
<td>BA</td>
<td>1.0±0.3†</td>
<td>4.3±0.6</td>
<td>2.5±0.4</td>
<td>83±8†</td>
<td>16.0±1.2*</td>
<td>107±4</td>
<td>33.7±1.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>1.2±0.2</td>
<td>4.6±0.4</td>
<td>2.0±0.6</td>
<td>112±15</td>
<td>17.4±2.7*</td>
<td>110±3</td>
<td>33.6±1.2</td>
</tr>
<tr>
<td>CBD</td>
<td>1.2±0.2</td>
<td>5.0±0.6</td>
<td>2.3±0.3</td>
<td>110±4</td>
<td>19.7±2.8*</td>
<td>106±2</td>
<td>33.7±1.1</td>
</tr>
</tbody>
</table>

Values are mean±SE; n=4. BA indicates baroreflex activation; BW, body weight; CBD, carotid body denervation; P<sub>ald</sub>, plasma aldosterone concentration; P<sub>cort</sub>, plasma cortisol concentration; P<sub>glu</sub>, plasma glucose concentration; P<sub>ins</sub>, plasma insulin concentration; P<sub>nep</sub>, plasma norepinephrine concentration; and PRA, plasma renin activity. *P*<0.05 vs control. †*P*<0.05 vs reduced fat.
Responses to CBD

Respiratory rate decreased from 12±1 to 8±1 breaths/min during the 18-hour postoperative period after CBD but on subsequent days returned to levels not significantly different from the elevated preoperative values associated with obesity (Figure 3). During the initial 4 postoperative days, there was fluid accumulation in the neck, resulting in occasional altered respirations and labored swallowing. These effects were largely subsided by day 5 postoperatively. To eliminate the potential influence of these untoward postoperative effects on the analysis for arterial pressure and heart rate, the initial 4 postoperative days were excluded from statistical evaluation of these variables. Mean arterial pressure on days 5 to 14 after CBD was significantly reduced when compared with the immediate preoperative hypertensive values (Figure 2). By day 14 of CBD, mean arterial pressure was reduced from 125±2 to 116±3 mmHg. Based on control values of 105 to 106 mmHg, this represents a 45% to 50% reduction in the severity of obesity-induced hypertension. After CBD, there were no significant changes in heart rate or in any other measured variables with the exception of blood gases.

As indicated in Table 2, prior to CBD, values for arterial partial pressure of CO₂ (PaCO₂) and pH in obesity were comparable to historical values in nonobese dogs. In marked contrast, PaO₂ was substantially depressed. Furthermore, after CBD, there were further substantial reductions in PaO₂ and a considerable increase in PaCO₂, consistent with successful CBD.

Breathing Rate Response to Baroreflex Activation in Nonobese Dogs

Despite chronically suppressing sympathetic activity, arterial pressure, and heart rate, chronic baroreflex activation did not lower respiratory rate in normotensive nonobese dogs (Figure 4).

Discussion

Although significant progress has been made in understanding the mechanisms whereby increased efferent sympathetic activity leads to obesity hypertension, the determinants of this heightened sympathetic activity in obesity remain uncertain. In this regard, there are several novel findings in this study. The significant and sustained antihypertensive effects of CBD, an especially important new finding, supports the hypothesis that hyperactivity of carotid body chemoreceptors contributes to the increase in arterial pressure in this experimental model of obesity hypertension. Additionally, these findings implicate hypoxemia as a likely primary stimulus that increases chemoreceptor activity with weight gain. Finally, a most impressive new observation was that chronic activation of the baroreflex has a powerful effect to counteract the central effects of chemoreceptor stimulation in obesity that lead to sympathoexcitatory, hypertension, and tachypnea.

Carotid Body Hyperactivity in Hypertension

Based on exaggerated sympathetic and ventilatory responses to acute activation of chemoreceptors by hypoxia in patients with hypertension, studies conducted several decades ago led to the speculation that chemoreceptor hyperactivity may contribute to forms of hypertension that are sympathetically mediated. This possibility is supported by additional acute observations showing that deactivation of carotid bodies with hyperoxia decreases efferent postganglionic muscle sympathetic nerve activity and arterial pressure in patients with essential hypertension and RSNA, arterial pressure, and ventilation in the SHR, responses that do not occur in the respective normotensive controls. However, until recently, the relevance of these acute observations to the pathogenesis of hypertension has not been critically tested.

The results of more recent studies strongly support the hypothesis that tonic activation of carotid chemoreceptors contribute to both the initiation and maintenance of hypertension in the SHR by increasing sympathetic activity. In a seminal study by Abdala et al., denervation of carotid chemoreceptors by sectioning the carotid sinus nerves bilaterally in prehypertensive SHRs prevented the full development of hypertension and appreciably attenuated established hypertension when the denervation was conducted in adult animals. In a follow-up investigation conducted in the adult SHR, McBryde et al. demonstrated that the chronic antihypertensive effects of bilateral carotid sinus ligation were associated with sustained suppression of RSNA, a response expected to lead to a chronic reduction in arterial pressure by increasing renal
excretory function. Despite the significance of these studies, the mechanism for tonic chemoreceptor activation in hypertension was not identified.

**Hypoxemia Drives Carotid Body Activation in Obesity Hypertension**

Given the above observations in the SHR, we hypothesized that tonic stimulation of the carotid bodies may contribute to obesity hypertension. This hypothesis was based on the premise that hypoxemia, the primary physiological stimulus for peripheral chemoreceptor activation, drives carotid body hyperactivity in obesity. The rationale for this is based on the observation that obesity is associated with increases in metabolic rate and oxygen consumption along with impaired respiratory mechanics. Excessive deposition of adipose tissue around the thoracic cage and in the abdomen limits lung expansion during inspiration and reduces lung volumes, especially expiratory reserve volume and functional reserve capacity.21–24 These changes in lung volume cause gas trapping and small airway narrowing/closure during expiration, leading to ventilation–perfusion mismatch and reduced PaO₂.21–24 Hypoxemia may be an underappreciated outcome of these abnormalities in patients with obesity because arterial oxygen hemoglobin saturation rather than PaO₂ is the more commonly measured variable. More specifically, arterial hemoglobin oxygen saturation may remain within the normal range, despite modest but significant hypoxemia, as reflected by the plateau region of the oxyhemoglobin dissociation curve. However, despite impaired ventilation and greater CO₂ production, most patients with obesity remain eucapnic because of compensatory increases in respiratory rate.21–24 The findings of hypoxemia (PaO₂=81±3 mmHg) and eucapnia (PaCO₂=39±0.3 mmHg) in the present study, in combination with increased respiratory rate, are consistent with these clinical observations. In a study conducted in 97 nonobese dogs, Haskins et al33 reported that PaO₂ and PaCO₂ were 100±1 mmHg and 40±1 mmHg, respectively (Table 2), values comparable to those reported by others in resting lean dogs.32,34 Although we did not measure blood gases during the control period before fat feeding, based on these historical measurements in nonobese dogs, we feel confident that the measured values for PaO₂ and PaCO₂ in this study during obesity reflect the occurrence of eucapnic hypoxemia. Therefore, these findings support our supposition that chronic hypoxemia is a likely stimulus for tonic activation of carotid bodies in obesity.

**Baroreflex Activation Abolishes Obesity Hypertension and Attenuates Tachypnea**

Studies in experimental animals and in human subjects have demonstrated that acute pressure-induced activation of the baroreflex attenuates chemoreceptor-mediated increases in sympathetic activity, peripheral resistance, and ventilation.10–12 The opposing effects of baroreceptor and chemoreceptor afferent input have been shown to include actions on the neurons within the nucleus tractus solitarius, the central site of termination of these afferent fibers.35 However, it is unclear whether the acute antagonism between the baroreflex and peripheral chemoreflex is relevant to long-term control of cardiovascular function. The ability to achieve chronically controlled increases in baroreceptor afferent activity

### Table 2. Arterial Blood Gas Determinations in Lean and Obese Dogs Before and After CBD

<table>
<thead>
<tr>
<th>Condition</th>
<th>PaO₂, mmHg</th>
<th>PaCO₂, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±1</td>
<td>40±0.3</td>
<td>7.38±0.01</td>
</tr>
<tr>
<td>Obesity</td>
<td>81±3</td>
<td>39±0.3</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>Obesity+CBD</td>
<td>65±2*</td>
<td>53±0.6*</td>
<td>7.37±0.03</td>
</tr>
</tbody>
</table>

*Values are mean±SE. Values in lean control dogs are taken from Haskins et al,33 n=97. Values in obesity and obesity+carotid body denervation (CBD), n=4. PaCO₂ indicates arterial partial pressure CO₂; and PaO₂, arterial partial pressure O₂. *P<0.05, obesity+CBD vs obesity.
by electric stimulation of the carotid sinus provides a unique experimental approach to evaluate the long-term implications of these acute observations. The sustained suppression of sympathetic activity and concomitant abolition of obesity hypertension by baroreflex activation in the present study emphasizes the importance of increased sympathetic activity in mediating this form of hypertension. Furthermore, in the context of the present study identifying increased peripheral chemoreflex activation as a stimulus that contributes to obesity hypertension, these findings are consistent with the hypothesis that one component of sustained baroreflex-mediated sympathoinhibition includes reduced chemoreceptor-mediated sympathoexcitatory drive through the central interactions of these reflexes. It is also possible that baroreflex activation may diminish the tonic excitatory effects of peripheral chemoreceptor activity in obesity by reducing sympathetic tone to the arterioles perfusing the carotid bodies. This would increase blood flow and suppress chemoreceptor activation by raising PaO₂ locally in the vicinity of the glomus cells or by reducing carotid body hyperactivity by other flow-dependent mechanisms.

Given that metabolic rate is increased in obesity, the finding of increased respiratory rate in this model of obesity hypertension is a new but rather predictable finding. A more significant novel observation was that the tachypnea of obesity was attenuated by baroreflex activation, whereas in nonobese dogs, no effect on respiratory rate was seen, despite appreciable baroreflex-induced blood pressure lowering and bradycardia (Figure 4). This suggests that the long-term antagonism between the baroreceptors and chemoreceptors on ventilation occurs only at heightened levels of chemoreceptor activation. In this regard, it is relevant that activation of carotid chemoreceptor afferent fibers by electric stimulation of the carotid sinus nerve may increase respiration and arterial pressure, changes opposite to those observed during carotid sinus stimulation in the obese dogs in the present study. However, despite carotid body afferents being in close proximity to carotid baroreceptors, there are no clinically relevant respiratory changes in hypertensive subjects with normal minute ventilation during electric stimulation of the carotid sinus at intensities that reduce arterial pressure.

Thus, electric stimulation of the carotid sinus seems to selectively activate carotid baroreceptors without costimulation of neighboring chemoreceptors. The present study does not provide any insight into whether the reduction in respiratory rate in obesity during carotid sinus stimulation reflects baroreflex-mediated antagonism of the chemoreflex by actions in the brain stem, an effect of baroreflex activation to diminish the hypoxic stimulation of carotid chemoreceptors by increasing carotid body perfusion, or some other response not readily apparent from the data on hand.

CBD Attenuates Obesity Hypertension

To investigate the role of carotid bodies in the maintenance of obesity hypertension, we abolished their central input by stripping the area around the carotid sinus rather than ligating the carotid sinus nerves. Because both approaches abolish central input from carotid baroreceptors as well as carotid chemoreceptors, elimination of carotid baroreflex-mediated inhibition of central sympathetic outflow may attenuate the reduction in sympathetic activity and the attendant fall in blood pressure after denervation of overactive carotid chemoreceptors. Despite the potential of this approach for underestimating the contribution of the carotid bodies to the hypertension, the hypertension in obese dogs was still reduced 45% to 50% after CBD. Therefore, this appreciable antihypertensive response to CBD indicates that tonic activation of carotid chemoreceptors contributes substantially to the neurogenically mediated hypertension associated with weight gain. Additionally, the time course of this antihypertensive response to CBD, beginning 4 to 5 days postoperatively, was similar to that reported in the SHR in which there were parallel reductions in arterial pressure and RSNA after bilateral carotid sinus denervation.

Accordingly, because bilateral renal denervation abolishes obesity hypertension in dogs, but does not lower arterial pressure in lean normotensive canines, it is likely that suppression of renal sympathetic outflow played a key role in mediating the antihypertensive response to CBD in the present study. Finally, it is relevant that denervation of the carotid sinus or ligation of the carotid sinus nerves does not lead to chronic changes in arterial pressure in normotensive, nonobese rats or dogs. Thus, in contrast to the contribution of hyperactive carotid chemoreceptors to the hypertension of obesity, the central input from the carotid bodies in normotensive subjects, in the absence of hypoxic activation, has no long-term influence on arterial pressure.

Although the direct effect of peripheral chemoreceptor activation includes a vagally mediated bradycardia, heart rate did not increase after CBD, consistent with the findings in SHRs with established hypertension. Because decreased vagal tone mediates the tachycardia of obesity, any influence on heart rate from sustained activation of the carotid chemoreflex is likely overridden by this primary mechanism.
The failure to measure significant reductions in plasma NE concentration after denervation of carotid chemoreceptors (Table 1) does not discount the probability that the antihypertensive response to carotid sinus denervation is mediated by suppression of sympathetic activity. For several reasons discussed by others, it is well established that measurement of plasma NE concentration is not sufficiently sensitive to capture relatively small but physiologically significant changes in overall sympathetic activity. This is evident by the absence of statistically significant increases in plasma levels of NE during the development of obesity hypertension in the present study (Table 1) or in obese subjects when compared with lean controls. Nonetheless, circulating levels of NE fell significantly during the pronounced suppression of central sympathetic outflow and arterial pressure during baroreflex activation. This response to baroreflex activation is consistent with our previous observations in obese dogs. In contrast, plasma levels of NE were unchanged after CBD. This regard, any sustained increases in sympathetic activity attributed to either denervation of carotid baroreceptors or pressure-induced unloading of aortic baroreceptors would be expected to attenuate the sympathoinhibition associated with loss of chemoreceptor hyperactivity, making it even more difficult to discern suppression of sympathetic activity by measurement of plasma NE concentration.

In keeping with the major function of the carotid bodies to sense hypoxemia and restore PaO2 to normal by increasing ventilation, previous studies indicate that these peripheral chemoreceptors play an important role in the regulation of respiration even in nonobese dogs during eupnea. The present study expands on these observations by providing comparative data in obesity hypertension. After elimination of the carotid body drive for ventilation by denervation of the carotid sinus, Rodman et al reported sustained reductions in respiratory rate and alveolar ventilation along with a decrease in PaO2 from 95±4 to 86±7 mm Hg and an increase in PaCO2 from 40±3 to 51±1 mm Hg during a 3-week observational period in nonobese dogs. In comparison, in the obese dogs of the present study, the corresponding changes in blood gases, particularly PaO2, were more pronounced as evident by a decrease in PaO2 from 81±3 to 65±2 mm Hg and an increase in PaCO2 from 40±3 to 53±0.6 mm Hg. These exaggerated changes in blood gases in obese dogs were even more remarkable in light of the progressive recovery in respiratory rate that followed the initial sharp reduction in the tachypnea after CBD. Using a similar technique for estimation of respiratory rate as in this study, Abdala et al also reported only a transient reduction in respiratory rate after CBD in SHR with established hypertension; however, because blood gases were not measured in their study, further comparison was not possible. Presumably, the recovery in respiratory rate in the present study was because of these more pronounced changes in PaO2 and PaCO2, providing more intense activation of peripheral aortic and central chemoreceptors. However, given the magnitude of the tidal flow/airway closure limitations and attendant ventilation/perfusion mismatch associated with obesity (as discussed above), the compensatory increase in respiratory rate attributed to stimulation of these other chemoreceptors was presumably insufficient to improve overall alveolar ventilation and restore CBD-induced changes in blood gases. However, without additional measurements of respiratory parameters, the current findings provide little insight into the mechanisms that lead to the quantitatively greater changes in PaO2 and PaCO2 after CBD in obese than in nonobese dogs. Nonetheless, these data indicate an especially important role of carotid body chemoreceptors in the regulation of ventilation with weight gain.

Limitation

We acknowledge that the number of dogs included in this study may have been too few to detect significant changes in all measured variables during baroreflex activation and after CBD, reflecting the possibility of a type II error. However, this is unlikely a major limitation in the present study for the following reasons: (1) responses to baroreflex activation and carotid sinus denervation were compared with control values in the same animal, minimizing the need for a larger data set, (2) experimental reproducibility through recovery from baroreflex activation (the first 56 days) was controlled by duplicating the protocol used in 2 of our previous studies in obese dogs, and the measured variables were comparable, and (3) the key novel findings in the last half of the current study, the marked fall in PaO2 and attenuation of obesity hypertension after CBD, were statistically significant. Parenthetically, the announcement by the National Institutes of Health that class B dogs could no longer be used for National Institutes of Health–supported research precluded acquiring additional class B dogs for this study.

Perspectives

Both electric stimulation of the carotid sinus and carotid body ablation are currently under investigation for the treatment of resistant hypertension. Although there is virtually no information from prospective studies or current clinical trials relating to the antihypertensive effects of carotid body ablation in humans, recent clinical trials have clearly shown that baroreflex activation reduces arterial pressure in many but not all patients with resistant hypertension. The reasons for this variability are not well understood, but because both baroreflex activation and carotid body denervation lower arterial pressure by inhibiting sympathetic activity, the variable degree of sympathetic activation in this heterogeneous population may be a key determinant of the antihypertensive response. Obesity-induced hypertension is mediated by activation of the sympathetic nervous system, and obesity is common in patients with resistant hypertension. Therefore, by showing that tonic activation of carotid chemoreceptors contributes significantly to the hypertension of obesity and that this chemoreceptor activation is associated with hypoxemia, the present study may provide a better understanding of the stimuli that contribute to sympathetic activation in resistant hypertension. However, it is noteworthy that although hypoxemia has been reported in some subjects with obesity, its overall prevalence in obesity is unclear. Perhaps a critical issue is the fat distribution pattern in obesity because impaired ventilatory function and gas exchange are better correlated with fat mass present around the thorax and in the abdominal cavity than with body mass index. That is, hypoxemia may be more likely to occur when fat impairs expansion of the chest and descent of
the diaphragm than when fat is devoid of mechanical effects on ventilatory function when present in the lower body cavity. If so, this may be one factor that accounts for the observation that visceral obesity elicits greater sympathetic activation than does subcutaneous obesity.31

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Disclosures
T.E. Lohmeier and E.D. Irwin have received consultant fees from CVRx, Inc. The other authors report no conflicts.

References

What Is New?
- We found in a clinically relevant canine model of obesity that tonic activation of carotid body chemoreceptors contributes to the sympathetically mediated hypertension. Hypoxemia seems to drive chemoreceptor hyperactivity.
- The excitatory effects of heightened chemoreceptor activity that lead to hypertension and increased respiratory rate are abolished by chronic electric stimulation of the carotid baroreflex.

What Is Relevant?
- Because obesity is common in resistant hypertension, the present study suggests that chemoreceptor hyperactivity may contribute to the variable degree of sympathetic activation and the antihypertensive response to rate in obesity-induced hypertension. *Am J Physiol Heart Circ Physiol*. 2013;305:H1080–H1088. doi: 10.1152/ajpheart.00464.2013.

Novelty and Significance

Hypoxemia is associated with weight gain in dogs fed a high-fat diet, suggesting that this may be a primary stimulus for increased chemoreceptor activation in obesity. The severity of obesity hypertension was reduced 45% to 50% by carotid body denervation and abolished by baroreflex activation, indicating that baroreflex-mediated sympathoinhibition can offset the central sympathoexcitatory effects of chemoreflex activation that promote hypertension. The hypoxemia of obesity is exacerbated by deafferentation of the carotid bodies.
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Chronic Interactions between Carotid Baroreceptors and Chemoreceptors in Obesity Hypertension

By

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Methods

Animal Preparation
Arterial and venous catheters were implanted for continuous measurement of arterial pressure and blood sampling, and for continuous intravenous infusion of isotonic saline as previously described.\(^1\)\(^-\)\(^3\) In a second surgery, a second generation miniaturized electrode (Barostim neo system) was sutured to the surface of each carotid sinus, and the lead bodies were tunneled subcutaneously and connected to a pulse generator implanted in the chest.\(^3\) The electrodes and the pulse generator for electrical stimulation of the carotid sinuses were provided by CVRx, Inc. (Minneapolis, MN).

After completion of the baroreflex activation protocol indicated below, a third surgery was performed to eliminate carotid body afferent activity into the central nervous system. Using the above protocol for anesthesia and postoperative analgesia, the carotid sinus electrodes were removed to expose the carotid sinuses and all tissue surrounding the common carotid artery and its external and internal branches was removed over a distance of 1-2 cm.\(^4\)\(^-\)\(^5\) Care was taken to not disrupt the arteries in close proximity to the common carotid and its branches. Finally the arteries and surrounding tissue were painted with 10% phenol in absolute alcohol to ensure disruption of all neural tissue.

General Methods for Experimentation During the Development and Maintenance of Obesity-Hypertension

Obesity hypertension was produced using the same protocol we previously reported.\(^6\)\(^-\)\(^7\) In short, during a 3-week postoperative period and throughout the study, the dogs were maintained in metabolic cages, given free access to water and fed a fixed daily diet containing ~5 mmol of sodium and ~55 mmol of potassium. In addition, the dogs received a continuous intravenous infusion of isotonic saline at a rate of 350 mL/day. Thus, total daily sodium intake was 55-60 mmol throughout the study. Water consumption was monitored daily and 24-hour urine samples were collected at 11 AM each day at the time of feeding. During the 3-week postoperative period, the dogs were trained to lie quietly in their cages for several hours each morning to allow arterial blood sampling. After this 3-week period of acclimation when electrolyte and fluid balance was achieved, steady-state control measurements were made. Subsequently, cooked beef fat was added to the regular diet for the remainder of the study. During the initial 35 days of the high-fat feeding, the diet was supplemented with 0.6 to 0.7 kg/day fat until body weight increased to ~137% of control. Once this weight gain was achieved, dietary fat was reduced (on day 36) to 0.1-0.15 kg/day to maintain a constant body weight for the remainder of the study. This reduction in fat intake commenced 5 days before electrical stimulation of the carotid baroreflex on day 41.

Estimation of Respiratory Rate Based on Respiratory Sinus Arrhythmia

Respiratory sinus arrhythmia is the most prominent component of the short term heart rate variability and thus identifiable as a distinct peak in the power spectrum of the pulse interval, centered at the respiratory frequency.\(^8\) Therefore, respiratory rate can be determined based on its impact on pulse interval oscillations using Fast Fourier Transform-based spectral analyses. However, Fast Fourier Transform inherently assumes periodicity, linearity and time-invariance of the biological signal, conditions largely unattainable in physiological conditions, especially when large time intervals (days) are considered. We therefore decided to use a simple
and straightforward time-domain analysis based on successive filtering of the daily time series. This method offers more flexibility and accuracy than the Fast Fourier Transform since it does not assume periodicity and allows minute to minute estimation of the respiratory rate. Furthermore, this method is amenable to use with large number of data, using commonly available software (Excel® and VBA® macro programing).

Data files originating from the blood pressure signal recorded continuously and sampled at 100 Hz (18 h long) were subjected to 3 consecutive moving window filters to extract successively local maxima (systolic blood pressure and dicrotic notch, first filter) and to generate a time series of systolic blood pressure and pulse interval (time difference between 2 successive peaks) (second filter) and finally respiratory rate (third filter). (Please see Figure 1 in main document). The moving window filters consisted of batches of \( n \) consecutive measurements (for the first filter \( n_1 = 9 \), for the second \( n_2 = 3 \) and the third \( n_3 = 7 \)). Application of the first filter was necessary in order to reduce the size of the data and accommodate the data limit for an Excel worksheet (~1 mil. lines). Therefore only local maxima are saved and used for further processing. The second filter was used to discriminate between local maxima generated by the systolic peak and dicrotic notch within the same heart period. The window sizes were optimized during initial tests. The central value within the moving window is identified as a minimum if it is smaller or equal than the rest of the numbers (for example first 3 and last 3 for \( n_3 = 7 \)). In the case when the sampling produced two consecutive low equal values, the second one is ignored to avoid counting a respiration twice. A rounded average of 3 minutes was used as a smoothing function. The average rate estimator for minute \( i \) was calculated as \( \bar{r}_i = (r_{i-1} + r_i + r_{i+1})/3 \), where \( r_i \) is the original respiration rate evaluated at minute \( i \).

**Results**

**Developmental Phase of Obesity Hypertension**

Respiratory rate increased during weight gain and was 12±1 breaths/min on the last day of high fat intake (day 34-35), compared to the control value of 8±1 breaths/min. Other than this variable not previously measured, all other hemodynamic, neurohormonal, and metabolic responses during the developmental phase of obesity were similar to those reported previously and are not detailed here. Rather, the values at the end of the high fat feeding (days-34-35) are summarized next and in Table 1. Along with weight gain to ~137% of control, there were increases in mean arterial pressure (MAP) from 106±3 to 124±3 mmHg and heart rate from 82±3 to 122±4 bpm. The induction of hypertension during weight gain was associated with marked sodium retention. During the control period, sodium and potassium excretion was 50±2 and 48±3 mmol/day, respectively, reflecting the intake of these electrolytes, whereas sodium excretion averaged 39±2 mmol/day during the 5 weeks on the high-fat diet. Thus, during the initial 35 days of the high-fat diet, there was an average retention of ~420 mmol of sodium. There was no significant change in potassium excretion during the high-fat diet.

The neurohormonal responses during the high fat diet were also similar to those we have reported previously. In brief, other than a transient increase in PRA during weeks 1-2 of the high fat diet, there were no statistically significant changes in the renin-angiotensin-aldosterone system or in plasma levels of cortisol and NE (Table 1). While plasma glucose concentration was unchanged, plasma insulin concentration was elevated throughout the entire 5 weeks of the high fat diet, indicating insulin resistance. In addition, there were no sustained changes in hematocrit or in the plasma concentrations of protein, sodium or plasma potassium during the development of obesity hypertension.
References