Sustained Activation of the Central Baroreceptor Pathway in Obesity Hypertension

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Abstract—The major goal of this study was to determine whether there is increased activation of medullary neurons that participate in the central baroreceptor reflex pathway in dogs with obesity-induced hypertension, a model of hypertension that is associated with increased sympathetic activity. We used Fos-like (Fos-Li) protein immunohistochemical methods to determine activation of neurons in the nucleus tractus solitarius (NTS), caudal ventrolateral medulla (CVLM), and rostral ventrolateral medulla (RVLM). Dogs were fed either a regular diet or an identical diet with the addition of 0.5 to 0.9 kg of cooked beef fat. After ∼6 weeks of the high fat diet, body weight (36.3±0.4 vs 21.5±0.5 kg), mean arterial pressure (105±4 vs 91±3 mm Hg), and heart rate (97±4 vs 70±3 bpm) were significantly greater in obese than in control dogs, respectively. There was little Fos-Li immunoreactivity in medullary neurons of control dogs but marked reactivity in obese dogs. Specifically, the number of Fos-Li–positive cells in the NTS and CVLM was 3 to 5 times greater in obese than in control dogs. Furthermore, despite sustained activation of these baroreceptor-sensitive neurons, there was a significantly greater number of Fos-Li positive cells in the RVLM of dogs fed the high fat diet. As baroreceptor suppression of sympathoexcitatory cells in the RVLM is mediated by activation of neurons in the NTS and CVLM, these results support recent findings indicating that baroreflex suppression of sympathetic activity is a long-term compensatory response in hypertension. However, sympathoexcitatory inputs onto RVLM neurons would appear to predominate over the inhibitory effects of the baroreflex in obesity hypertension. (Hypertension. 2003;42:96-102.)

Key Words: angiotensin ■ hypertension, obesity ■ baroreflex ■ central nervous system ■ brain

There is considerable evidence that activation of the sympathetic nervous system plays an important role in the pathogenesis of hypertension.1–3 However, the factors that chronically influence sympathetic activity and the precise mechanisms that mediate neurally induced hypertension are unclear. In large part, this has been due to technical limitations that prevent assessment of sympathetic function under chronic conditions. An area of long-standing interest, but one of considerable uncertainty, concerns the potential impact of baroreflexes on sympathetic activity and arterial pressure in hypertension. Clearly, baroreflex function is often impaired in chronic hypertension,4 but whether baroreflex dysfunction contributes to increased sympathetic activity and the severity of hypertension is unresolved.

The hypothesis that baroreflex dysfunction contributes to increased sympathetic activity in hypertension implies that baroreflexes normally play a role in the chronic (as well as the acute) regulation of body fluid volumes and arterial pressure. On one hand, a major argument against this possibility is that baroreflexes reset in the direction of the prevailing level of arterial pressure.5 On the other hand, although chronic resetting is a universal finding, the magnitude and time course of resetting is not clearly established. Moreover, a number of recent observations in chronically instrumented animals indicate that baroreflex resetting is incomplete in hypertension.6–13 Additionally, these studies indicate that baroreflexes chronically suppress renal sympathetic nerve activity and promote sodium excretion in hypertension. As alterations in renal excretory function play a critical role in the long-term regulation of arterial pressure, this suggests that baroreflex suppression of renal sympathetic nerve activity represents a chronic compensatory response in hypertension.

Over the last decade, the method of immunohistochemical labeling of Fos, the protein product of the immediate early gene c-fos, has been used widely to identify central neurons involved in the regulation of arterial pressure.14 Studies employing Fos immunohistochemistry have clearly identified both medullary neurons of the baroreflex and other central neurons that are activated in response to acute increases in arterial pressure induced by pressor agents.11,14–17 However, relatively few studies have used this methodology to determine sites of neuronal activation in chronic hypertension. More specifically, Fos-like (Fos-Li) immunohistochemistry has not been used previously to identify excitatory and...
inhibitory neurons activated in obesity hypertension, a particularly prevalent form of human hypertension.

Our previous results using Fos-Li immunochemistry indicate that there is sustained activation of the central baroreflex pathway in angiotensin (ANG II) hypertension, a finding consistent with our earlier studies indicating that the baroreflex accounts for chronic renal sympathoinhibition in ANG II hypertension.6–11 Although these studies of ANG II hypertension lasted up to 10 days, they still leave unanswered the question of the influence of the baroreflex on sympathetic activity in more long-standing hypertension. We hypothesized that if the baroreflex truly has an important influence on sympathetic activity in chronic hypertension, then the canine model of obesity hypertension, induced by feeding dogs a high fat diet for several weeks, should be associated with increased activation of neurons that are part of the central baroreflex pathway. This clinically relevant model of human hypertension would appear to be an interesting test of the above hypothesis because the renin-angiotensin system is activated, but unlike the hypertension associated with chronic ANG II infusion, obesity hypertension is not associated with suppression of renal sympathetic nerve activity. In fact, there is strong evidence, both in experimental animals and in human subjects, that renal sympathetic nerve activity is increased in obesity hypertension.1,3,18–19 These results could be interpreted to indicate that in obesity the baroreflex wanes in the face of long-standing hypertension permitting the expression of the sympathoexcitatory influences of ANG II and other factors.

Therefore, we used Fos-Li immunohistochemistry to determine the expression of Fos and Fos-related antigens in the medulla of dogs subjected to obesity-induced hypertension. In addition to emphasizing the activity of neurons comprising the baroreflex, we also examined Fos-Li immunoreactivity in several central regions that potentially mediate the sympathoexcitatory effects of hormones and neuropeptides associated with ANG II and obesity hypertension.3,20–25

Methods

Animal Preparation
All procedures were performed in accordance with National Institutes of Health Guidelines and approved by the Institutional Animal Care and Use Committee. Twelve male dogs with an initial weight of 20.5 to 23.5 kg each were used in this study. Catheters made of Tygon microbore tubing were implanted in the lower abdominal aorta and inferior vena cava and were exteriorized between the scapulae.7–11 The procedures for daily maintenance of the dogs have been described previously.7–11

Experimental Protocol
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 ‘5 mmol of sodium and 5 mmol of potassium. Dogs were fed at 6–11 AM. They were also continuously infused intravenously with isotonic saline at a rate of ∼400 mL/day. Thus, sodium intake was ∼65 mmol/day. In addition, cooked beef fat (0.5 to 0.9 kg/day) was added to the regular diet of 6 of the dogs for ∼6 weeks. During this time, these dogs and the dogs fed only the regular diet were trained to lie quietly in their cages in the morning. Only a technician remained in the room with the dogs during these morning sessions.

Between 9 and 10 AM on the morning of the terminal experiment, the dogs were placed in a recumbent position on the cage floor for no less than 60 minutes. The values illustrated in Figure 1 represent the mean arterial pressure (MAP) and heart rate (HR) in control dogs and in dogs fed a high fat diet. Values are mean ± SEM (n=6 for each group). *P<0.05 versus control. bpm indicates beats per minute.

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Figure 1. Body weight (BW) and resting values for mean arterial pressure (MAP) and heart rate (HR) in control dogs and in dogs fed a high fat diet. Values are mean ± SEM (n=6 for each group). *P<0.05 versus control. bpm indicates beats per minute.

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Fos Immunohistochemistry
Free-floating sections from each brain were processed for Fos immunohistochemistry using an avidin-biotin peroxidase procedure as previously described.11,12 In the present study, a c-Fos polyclonal antibody (K-25, sc-253, Santa Cruz Biotechnology Inc) was used. This antibody reacts with other Fos-related antigens including Fos B, Fra-1, and Fra-2. This antibody has been used previously by us and others to examine long-term changes in Fos-Li staining in hypertensive animals.11–26 The anti-Fos antibody was diluted 1:400 in PBS, and the sections were incubated in the primary antibody at 4°C for 72 hours. On completion of all reactions, the sections were placed on gelatin-coated slides, dried, and cover-slipped.

Microscopic Analysis and Quantification
The sections were examined using light microscopy to identify Fos-Li–stained cells in central neurons subserving the baroreflex, as well as in forebrain and hindbrain regions believed to be important in integrating neurohormonal activation in obesity hypertension. Specifically, the regions examined were the nucleus tractus solitarius (NTS), caudal ventrolateral medulla (CVLM), rostral ventrolateral medulla (RVLM), area postrema (AP), paraventricular nucleus (PVN), and supraoptic nucleus (SON). Additionally, as a control for noncardiovascular-related neuronal activation in obesity, Fos-Li staining was also determined in the spinal trigeminal nucleus, which is located lateral to the NTS/AP complex. Digital images from all regions of interest were recorded using an Olympus IX50 micro-
Fos-Li Immunoreactivity in the Medulla

The photomicrographs in Figure 2 illustrate the darkly stained nuclei in cells of the NTS exhibiting Fos-Li immunoreactivity. In the NTS, as in other regions of the medulla, there were few Fos-Li–positive cells in control dogs. In contrast, there were distinctly more Fos-Li–positive cells in the NTS of obese than control dogs. These cells were located in regions of the NTS that have been previously reported to contain baroreceptor-sensitive neurons.28

Figure 3 summarizes the number of Fos-Li neurons/section in various regions of the medulla in lean and obese dogs. In control dogs, Fos-Li staining was low in all brain regions as expected. In obese dogs, the number of Fos-Li–positive cells was significantly increased compared with control dogs in areas of the medulla that are part of the central baroreflex pathway. Specifically, in the NTS and CVLM, there were ~3 to 5 times more Fos-Li–positive cells in obese than in lean dogs. However, despite activation of these baroreceptor-sensitive neurons, there was also a significant, albeit much
RVLM. Fos-Li staining was increased in the RVLM in the
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pattern of central neurons in obesity and ANG II hypertension
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Fos-Li immunoreactivity to investigate the central baroreflex
pathway in obesity hypertension. This supports the concept, based on a number
of complementary techniques in chronically instrumented animals, that the baroreflex has a sustained suppressive influence on sympathetic activity in hypertension. Additionally, the current results indicate that the activation pattern of central neurons in obesity and ANG II hypertension is similar, with one notable exception: the neurons of the RVLM. Fos-Li staining was increased in the RVLM in the
obese dogs of the present study but not in dogs chronically
infused with ANG II. Increased Fos-Li expression in the
RVLM of obese dogs supports the observations that sympathetic activity to the kidneys and other vascular beds is increased in obesity hypertension. In contrast, the absence of increased Fos-Li staining in the RVLM in ANG II hypertension is consistent with recent findings indicating that
renal sympathetic nerve activity is suppressed during chronic ANG II infusion.

Because of the inherent difficulties in achieving chronic nerve recordings that faithfully provide accurate quantitative time-dependent changes in sympathetic activity, it is unknown to what extent baroreflex control of sympathetic activity may adapt in chronic hypertension. Acute studies have demonstrated that baroreflex inhibition of sympathetic activity is impaired in obesity and in obesity hypertension. However, if baroreceptors undergo rapid adaptation and complete resetting, baroreflex dysfunction would appear to have little relevance to the pathogenesis of obesity hypertension because baroreflexes would be unable to participate in long-term regulation of sodium excretion and arterial pressure. Thus, a number of recent observations in chronically instrumented animals are particularly significant because they strongly support the hypothesis that baroreflexes do not totally reset in chronic hypertension. Regarding ANG II hypertension, these studies indicate that baroreflexes chronically suppress renin sympathetic nerve activity during hypertension produced by up to 10 days of ANG II infusion.

We reasoned that obesity-induced hypertension in dogs would provide a critical test of this concept and would be an especially relevant model of hypertension to study for several reasons. First, the association of obesity and hypertension is prevalent in human subjects, and dogs fed high fat diets have many characteristics of obese humans, including similar sympathetic, endocrine, renal, cardiovascular, and metabolic changes. Further, although the precise mechanisms that account for obesity hypertension are unclear, sympathetic activation appears to play an important role. Second, obesity hypertension is associated with activation of the renin-angiotensin system, raising the possibility that ANG II may contribute to increased sympathetic activity in obesity. Therefore, comparing patterns of neuronal activation associated with high endogenous plasma levels of ANG II in obese dogs with patterns in dogs made hypertensive by continuous intravenous infusion of ANG II might provide greater insight into the mechanisms that account for central activation of the sympathetic nervous system in obesity. Third, as mentioned above, there are diagnostically opposite changes in renal sympathetic nerve activity in obesity and ANG II hypertension, and yet both models of hypertension are associated with elevated plasma levels of ANG II, a hormone that increases sympathetic activity. Therefore, one could interpret the increased renal sympathetic activation characteristic of long-standing obesity hypertension to reflect, at least in part, waning of the sympathoinhibitory effects of the baroreflex during the evolution of the hypertension. However, if the neurons in the central baroreflex pathway are chronically activated, this would fail to support this hypothesis.

To test whether there is increased activation of the baroreflex in obesity hypertension, we used Fos methodology to determine the activity of central neurons involved in the baroreflex. One advantage of this methodology over the use of electrophysiological techniques is that it can be used to map a large number of activated neurons, rather than the activity in individual neurons. Another important advantage is that Fos methodology can be used in animals that remain smaller, increase (≈65%) in Fos-Li-positive cells in the
RVLM of dogs fed the high fat diet. Additionally, the number of Fos-Li-positive cells in the AP was ≈3 times greater in obese than in lean dogs. Finally, there was no significant difference in Fos-Li staining in the spinal trigeminal nucleus of lean and obese dogs.

Fos-Li Immunoreactivity in the Hypothalamus
As illustrated in Figure 4, Fos-Li staining was also significantly increased in the supraoptic (≈5×control) and paraventricular nuclei of dogs with obesity-induced hypertension. In obese dogs, the increase in Fos-Li-positive cells was considerably greater in the parvocellular paraventricular nucleus (≈4×control) than in the magnocellular paraventricular nucleus (≈2×control).

Discussion
To the best of our knowledge, this is the first study to use Fos-Li immunoreactivity to investigate the central baroreflex pathway in obesity hypertension. The most important finding of the present study is that the neurons subserving the baroreflex appear to be chronically activated in obesity hypertension. This supports the concept, based on a number of electrophysiological techniques in chronically instrumented animals, that the baroreflex has a sustained suppressive influence on sympathetic activity in hypertension. Additionally, the current results indicate that the activation pattern of central neurons in obesity and ANG II hypertension is similar, with one notable exception: the neurons of the RVLM. Fos-Li staining was increased in the RVLM in the obese dogs of the present study but not in dogs chronically infused with ANG II. Increased Fos-Li expression in the RVLM of obese dogs supports the observations that sympathetic activity to the kidneys and other vascular beds is increased in obesity hypertension. In contrast, the absence of increased Fos-Li staining in the RVLM in ANG II hypertension is consistent with recent findings indicating that

![Figure 4. Number of Fos-Li neurons per section in various regions in the hypothalamus in control dogs and in dogs fed a high fat diet. Values are mean±SEM (n=6 for each group).](image-url)
conscious during the induction of hypertension, and therefore, we circumvent the confounding influence of anesthesia and surgical stress on neuronal activation. To reduce the possible effects of general excitement on Fos-Li expression in the present study, experiments were conducted under resting conditions, and only after the dogs were thoroughly conditioned to their home environment. Further, to minimize the potential influence of variations in body fluid volumes and arterial pressure on neuronal activation, independently of obesity, the dogs were maintained on a constant salt and water intake by continuous infusion of isotonic saline. Additionally, they were fed an identical diet, with the exception of the added beef fat. The low levels of Fos-Li immunoreactivity in the control group indicate that these experimental concerns were adequately addressed. Because of the fact c-Fos expression in response to a number of stimuli appears to be rather transient, we were uncertain whether measurement of c-Fos alone would be an appropriate marker for neuronal activation in the present chronic study. In contrast to c-Fos, the expression of Fos-related proteins persists during chronic neuronal activation. Therefore, we used an antibody that reacts not only with c-Fos, but also with Fos family proteins. This antibody has been used previously by us and others to identify central neurons activated in hypertension.

Our study is the first to determine the activity of neurons in the central baroreceptor pathway during chronic obesity-induced hypertension. It is well established that spinally projecting neurons in the RVLM provide tonic excitatory drive to sympathetic preganglionic neurons that control sympathetic output to the peripheral circulation. It is also recognized that activation of neurons in the NTS, the site of termination of baroreceptor inputs, and their subsequent activation of inhibitory neurons in the CVLM, play an important role in mediating baroreflex inhibition of sympathetic cells in the RVLM. This concept is corroborated by increased Fos staining in the NTS and CVLM during acute elevations in arterial pressure in animals with intact baroreflexes and the absence of such increase in animals without baroreflexes. Therefore, the most important and novel finding in the present study is that this same pattern of activation of NTS and CVLM neurons, which we and others have reported during acute stimulation of the baroreflex, persists during chronic obesity hypertension. This suggests that there is sustained activation of the baroreflex during obesity hypertension and so provides complementary data to support the contention that baroreflex-mediated sympathoinhibition is a long-term compensatory response in this and in other forms of hypertension. However, despite sustained activation of the baroreflex pathway, there was a smaller, but still statistically significant, increase in the number of Fos-Li cells in the RVLM of dogs with obesity hypertension. This change suggests that other excitatory inputs into the RVLM may predominate over the chronic inhibitory effects of the baroreflex. Further, because the number of Fos-Li cells is not increased in the RVLM in dogs made hypertensive by chronic infusion of ANG II, it would appear that stimuli other than ANG II are essential for this activation of RVLM neurons in obesity hypertension.

The PVN of the hypothalamus, composed of both magnocellular and parvocellular neurons, is an important site for autonomic and neuroendocrine regulation. In the present study, there was a substantial increase in Fos-Li staining in the PVN of obese dogs. PVN neurons receive and integrate ascending signals from hindbrain regions related to pressure and volume control and signals from forebrain regions including the circumventricular organs of the lamina terminals, an area that transduces the excitatory effects of circulating ANG II. The centrally mediated responses to circulating ANG II include activation of the sympathetic nervous system, stimulation of vasopressin secretion, and stimulation of thirst. The parvocellular PVN also plays an important role as an integrating center regulating food intake and body weight with satiety centers in the brain stem and is believed to be part of the central pathway that mediates the anorexic and sympathoexcitatory actions of leptin. Additionally, leptin has received considerable attention as a possible factor contributing to sympathoexcitation and hypertension in obesity. Projections from the PVN do extend to the RVLM and sympathetic preganglionic neurons of the spinal cord, and activation of PVN neurons increases sympathetic outflow to the kidneys. Therefore, it is possible that the increased Fos-Li staining in PVN neurons of obese dogs reflects sympathoexcitatory actions of ANG II and/or leptin that lead to the hypertension. However, additional studies are needed to substantiate this possibility, particularly because the different populations of PVN neurons have a multitude of cardiovascular and noncardiovascular targets.

Additional studies are also needed to address the significance of increased Fos-Li staining in other cells of the hypothalamus and medulla. In obese dogs, an increase in Fos-Li staining was particularly prominent in the magnocellular neurons of the PVN and SON. Similarly, an increase in Fos-Li staining in magnocellular neurons also occurred in dogs made hypertensive by chronic infusion of ANG II. However, these dogs do not have increased plasma levels of vasopressin. This raises the interesting possibility that the increase in Fos-Li staining in magnocellular neurons reflects activation of oxytocinergic neurons. Because there is some evidence that physiological increments in plasma levels of oxytocin are natriuretic, a persistent increase in oxytocin secretion in obesity could provide a compensatory mechanism to attenuate the severity of hypertension. Dual immunostaining of Fos-Li-positive cells for AVP and oxytocin, along with measurement of plasma levels of these neurohypophysial hormones in obese dogs, would provide some insight into this question.

Dogs with obesity hypertension, like dogs chronically infused with ANG II, also had increased Fos-Li staining in the AP. The AP is a circumventricular organ that appears to be important in mediating the sustained sympathoexcitatory effects of ANG II. Therefore, it is possible that the elevated plasma levels of ANG II present in obesity could contribute
to increased sympathetic activation and hypertension by actions initiated at the AP.

Finally, it should be acknowledged that the major conclusion of this study—that the baroreflex pathway is chronically activated in obesity hypertension—is based on Fos-Li staining in discrete areas of the medulla previously described as composing the baroreflex and impacting sympathetic activity and arterial pressure in anesthetized dogs. However, it was not feasible in these conscious dog experiments to provide additional evidence that the Fos-Li–positive cells were barosensitive. Thus, the results of this study should be carefully interpreted with this caveat in mind. Nonetheless, as the quantification of Fos-Li–positive cells was based on precise neuroanatomical landmarks,27–29 we feel it is highly likely that the increased Fos-Li staining in the NTS, CVLM, and RVLM of obese dogs reflected activation of neurons in the baroreflex pathway. Additionally, the activation pattern of neurons in the NTS and CVLM, presumably reflecting baroreflex suppression of sympathoexcitatory RVLM neurons, was essentially the same as we reported previously in conscious dogs during elevations in arterial pressure produced by both acute and chronic ANG II infusion.11 Further, the absence of increased Fos-Li staining in the spinal trigeminal nucleus, which is lateral to the NTS/AP complex and is not directly involved in cardiovascular control, suggests that increased Fos-Li staining in the NTS and CVLM was not a nonspecific response associated with obesity. Thus, the challenge in future studies will be to further elucidate the significance of the Fos-Li expression patterns in the medulla and hypothalamus of obese dogs using techniques that do not require anesthesia and surgical stress.

Perspectives

There is considerable evidence that the sympathetic nervous system is activated in obesity and plays a causal role in the pathogenesis of hypertension. More specifically, increased renal sympathetic nerve activity appears to be critically involved in mediating obesity hypertension. Although the basis for sympathetic activation is unresolved, much attention has been directed toward the central sympathoexcitatory actions of ANG II and leptin. By providing further support for the notion that the baroreflex is chronically activated in hypertension, the present findings suggest another mechanism for increased sympathetic activity in obesity: baroreflex dysfunction. Because the central actions of both ANG II and leptin that increase sympathetic activity are barosensitive,9,40,41,42 the progressive impairment of baroreflex control of sympathetic activity during the evolution of obesity hypertension may be particularly significant in contributing to further increments in sympathetic activity and arterial pressure in the more advanced stages of obesity hypertension.4

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