Obstructive Sleep Apnea

An Essential Role for ΔFosB in the Median Preoptic Nucleus in the Sustained Hypertensive Effects of Chronic Intermittent Hypoxia

J. Thomas Cunningham, W. David Knight, Steven W. Mifflin, Eric J. Nestler

Abstract—One of the main clinical features of obstructive sleep apnea is sustained hypertension and elevated sympathetic activity during waking hours. Chronic intermittent hypoxia (CIH), animal model of the hypoxemia associated with obstructive sleep apnea, produces a similar sustained increase in blood pressure. This study determined the role of ΔFosB in the median preoptic nucleus (MnPO) in the sustained increase in mean arterial pressure associated with CIH. Rats were injected in the MnPO with viral vectors that expressed green fluorescent protein alone or green fluorescent protein plus a dominant-negative construct that inhibits the transcriptional effects of ΔFosB. In green fluorescent protein–injected rats and uninjected controls, 7-day exposure to CIH increased mean arterial pressure by 7 to 10 mm Hg during both intermittent hypoxia exposure and normoxia. Dominant-negative inhibition of MnPO ΔFosB did not affect changes in mean arterial pressure during intermittent hypoxia exposure but significantly reduced the sustained component of the blood pressure response to CIH during the normoxic dark phase. Inhibition of MnPO ΔFosB reduced the FosB/ΔFosB staining in the paraventricular nucleus and rostral ventrolateral medulla but not the nucleus of the solitary tract. PCR array analysis identified 6 activator protein 1–regulated genes expressed in the MnPO that were increased by CIH exposure, ace, ace2, nos1, nos3, prdx2, and map3k3. Dominant-negative inhibition of ΔFosB in the MnPO blocked increased expression of each of these genes in rats exposed to CIH except for Prdx2. ΔFosB may mediate transcriptional activity in MnPO necessary for sustained CIH hypertension, suggesting that neural adaptations may contribute to diurnal hypertension in obstructive sleep apnea. (Hypertension. 2012;60:179-187.) ● Online Data Supplement

Key Words: hypertension ■ sleep apnea ■ angiotensin ■ hypothalamus ■ sympathetic nervous system

Sleep apnea commonly refers to a number of disorders that are characterized by repetitive bouts of disordered breathing leading to interruptions or reductions in airflow during sleep.1,2 The episodic hypoxemia resulting from sleep-disordered breathing has been proposed to produce cardiovascular and metabolic diseases in patients with sleep apnea, and chronic activation of the sympathetic nervous system is central to the development of cardiovascular disease associated with these disorders.1–3 Studies of chronic intermittent hypoxia (CIH) in animal models have identified several factors that contribute to this chronic activation of the sympathetic nervous system and resulting hypertension that include sensitization of carotid chemoreceptors and activation of the renin-angiotensin system.4–7 Recent studies indicate a role for the central nervous system in the sustained hypertension and sympathetic activation associated with CIH.5–10 However, the central nervous system (CNS) regions and mechanisms involved have not been fully determined.

FosB and its more stable splice variant ΔFosB are members of the activator protein (AP) 1 transcription factor family that are expressed in the CNS after repeated activation of the CNS.11–13 In a previous study, we exposed adult male rats to CIH for 7 days and used ΔFosB immunohistochemistry to identify regions that were chronically activated by this protocol. CIH increased ΔFosB staining in CNS regions that regulate sympathetic outflow, including the lamina terminalis, paraventricular nucleus of the hypothalamus (PVN), rostral ventrolateral medulla, and nucleus of the solitary tract.14

The lamina terminalis consists of 3 separate regions that lie along the anterior wall of the third ventricle, the subfornical organ, median preoptic nucleus (MnPO), and organum vasculosum of the lamina terminalis. Both the subfornical organ and organum vasculosum of the lamina terminalis are circumventricular organs that lack a functional blood-brain barrier and contain neurons that are sensitive to changes in
plasma osmolality and to circulating peptides, including angiotensin II. 15–17 Along with the MnPO, these regions are critically involved in central autonomic regulation and body fluid homeostasis. 17–20 In animal models, electrolytic lesions of the ventral lamina terminalis (anteroventral region of the third ventricle [AV3V]) reverse or prevent many forms of neurogenic hypertension, 21 so we tested the effects of electrolytic AV3V lesions on CIH hypertension. Based on our results, we conducted additional experiments to further characterize the contribution of this region and the role of chronic transcriptional activation of the MnPO in CIH hypertension. Several transcription factors have been identified as candidates for mediating the effects of intermittent hypoxia on gene expression related to changes in sympathetic outflow including the AP-1 family of transcription factors. 22 Studies using virally mediated dominant-negative inhibition of ΔFosB have shown that it regulates changes in gene expression that are necessary for several behavioral adaptations after repeated exposure to drugs of abuse, stress, or antidepressant treatments. 23–27 We conducted experiments to determine whether ΔFosB plays a similar role in the lamina terminalis during CIH. These experiments focused on the MnPO because it is highly interconnected with both the subfornical organ and organum vasculosum of the lamina terminalis and has a major efferent projection to the PVN. 28 Also, ibotenic acid lesions of the MnPO have been shown to attenuate the sustained phase of angiotensin II–induced hypertension in the rat. 29 To test this hypothesis, an adeno-associated viral vector (AAV) that expresses ΔJunD, a dominant-negative construct that antagonizes ΔFosB- and other AP-1–mediated transcription, was stereotaxically injected in the MnPO region before 7 days of CIH exposure. Using radiotelemetry recording to measure changes in blood pressure, heart rate (HR), and respiratory rate (RR), we observed that dominant-negative inhibition of MnPO ΔFosB attenuated the sustained component of the CIH pressor response and altered the pattern of ΔFosB staining in CNS autonomic regulatory regions. We also combined virally induced dominant-negative inhibition of MnPO ΔFosB with PCR array analysis to identify several AP-1–regulated genes that could mediate the contribution of the MnPO to CIH-induced hypertension.

Methods

Animals

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; 250–350 g) were individually housed and maintained on a 14:10-hour light cycle and provided with ad libitum access to food and water. Animal procedures were conducted according to National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee.

Electrolytic AV3V Lesions

Rats were anesthetized with isofluorane (2%) and placed in a stereotaxic instrument equipped to deliver isofluorane anesthesia. A 23-gauge nichrome electrode (insulated except at the tip) was placed 0.2-mm posterior and 7.5-mm ventral from bregma at the midline, and anodal current (2.5 mA) was applied for 20 seconds. The same electrode was used for sham lesions but it was placed 6.5 mm ventral to bregma. Each rat was monitored for adipsia after surgery. Lesioned rats that displayed adipsia were provided with 10% sucrose to drink and weaned to tap water as described previously. 30 Lesioned rats were only included in the study if they demonstrated adipsia immediately after surgery. After 2 weeks of recovery, the rats were instrumented with radiotelemetry transmitters.

Virally Mediated Dominant-Negative Inhibition of MnPO ΔFosB

For MnPO injections, rats were anesthetized with isofluorane (2%) and placed in a stereotaxic head frame equipped with an isofluorane delivery system (David Kopf Instruments, Tujunga, CA). A midline incision was made through the disinfected scalp and the skull exposed around bregma 31 with the injector angled 8° from vertical. Each 400-nL injection was delivered through a 30-gauge stainless steel injection needle connected to a Hamilton 5-μL syringe. Rats were treated with constructs expressing green fluorescent protein (GFP) only (AAV-GFP) or expressing GFP and ΔJunD (AAV-GFP-ΔJunD). Vectors were constructed and packaged as described previously and titers of the vectors averaged 2.0×10^7 infectious units/mL. 23–25 Injections were made 2 weeks before telemetry surgery.

Telemetry Monitoring of Blood Pressure and HR

A Dataquest IV radiotelemetry system (Data Sciences Inc, St Paul, MN) was used to continuously record mean arterial pressure (MAP), HR, RR, and activity. Using gas anesthesia (isofluorane 2%), rats were implanted with an abdominal aortic catheter attached to a TA11PA-C40 radiotelemetry transmitter. The transmitter was secured to the abdominal muscle and remained in the abdominal cavity for the duration of the experiment. Two weeks were allowed for recovery from surgery. Blood pressure measurements obtained during a 10-second sampling period (500 Hz) were averaged and recorded every 10 minutes. Signals from radiotransmitters were measured at atmospheric pressure preoperatively and postoperatively to quantify and correct for any signal drift over the course of the protocol. In most cases, no postmortem offset adjustments were required.

CIH Protocol

After postsurgical recovery, rats individually housed in their home cages were relocated into custom-built Plexiglas chambers 1 week before beginning the treatment period, as described previously. 14,32 Rats were allowed to acclimate to the chambers at normoxia (21% O2) for 4 days before recording baseline cardiovascular data for 3 days. Theretofore, rats were exposed to CIH for 7 days from 8:00 am to 4:00 pm. The O2 concentration in the chambers was regulated using custom-built user-controlled timers that separately switched the flow of room air and nitrogen into each chamber. Flow rates of room air and nitrogen to each chamber were separately controlled using individual flow meters (University of Texas Health Science Center at San Antonio Instrumentation Services). The CIH protocol was as follows: (1) O2 was reduced from 21% to 10% in 105 seconds; (2) O2 was held at 10% for 75 seconds; (3) O2 was returned to 21% in 105 seconds; and (4) O2 was held at 21% for 75 seconds. Each complete cycle lasted 6 minutes, and the rats were exposed to 80 cycles per day. Chambers were maintained at 21% throughout the remainder of the light phase (4 hours) and the dark phase (10 hours).

Immunohistochemistry and Histology

The morning after the last CIH exposure, rats were anesthetized with thioptabarbital (Inactin, Sigma, 100 mg/kg IP) and were perfused transcardially with PBS followed by 4% paraformaldehyde. Brains were postfixed for 1 to 2 hours followed by cryoprotection in 20% sucrose (PBS) at 4°C. Three sets of coronal 40-μm sections from each brain were preserved in cryoprotectant and stored at −20°C to be processed at a later time. Separate sets of serial sections from each rat were processed for FosB (goat anti-FosB, Santa Cruz Biotechnology, Santa Cruz, CA; 1:1000) immunohistochemistry, as described previously or used to...
verify the location of the injections sites. The anti-FosB antibody used in this study does not discriminate full-length FosB from the splice variant ΔFosB.33 Sections were then processed with a biotin-conjugated horse antigoat IgG (Vector Laboratories, Burlingame, CA; 1:200) and reacted with an avidin-peroxidase conjugate (Vectastain ABC kit; Vector Laboratories) and PBS containing 0.04% 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfite for 10 to 11 minutes.

Sections were analyzed using an Olympus microscope (BX41) equipped for epifluorescence and an Olympus DP70 digital camera with DP manager software (version 2.2.1). Images were uniformly adjusted for brightness and contrast. Regions were identified using the rat brain stereotaxic atlas of Paxinos and Watson.33 FosB/ΔFosB-positive cells in each region were counted using ImageJ. Four to 6 sections were analyzed from each rat for each region.

**Real-Time Quantitative PCR Array**

Each rat was anesthetized with Inactin (100 mg/kg IP) and euthanized. Their brains were removed and punch samples were collected containing the MnPO, as described previously.34 RNA was isolated from the punch samples using TRIzol reagent following the manufacturer’s instructions. RT-PCR was performed using the manufacturer’s protocol (Invitrogen, Carlsbad, CA) and dissolved in Tris/EDTA buffer. Reverse transcription of cDNA was performed according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA; 1:200) and reacted with an avidin-peroxidase conjugate (Vectastain ABC kit; Vector Laboratories) and PBS containing 0.04% 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfite for 10 to 11 minutes.

Sections were analyzed using an Olympus microscope (BX41) equipped for epifluorescence and an Olympus DP70 digital camera with DP manager software (version 2.2.1). Images were uniformly adjusted for brightness and contrast. Regions were identified using the rat brain stereotaxic atlas of Paxinos and Watson.33 FosB/ΔFosB-positive cells in each region were counted using ImageJ. Four to 6 sections were analyzed from each rat for each region.

**Figure 1.** Electrolytic lesions of the anteroventral region of the third ventricle (AV3V) region did not affect the increase in mean arterial pressure (MAP) during intermittent hypoxia exposure (A) but was associated with a significant decrease in MAP during the dark phase (B). (C) Chronic intermittent hypoxia (CIH) sham (n = 5); (D) CIH AV3V (n = 6). Differences in heart rate between the 2 groups were not observed for either time period (C and D). *A significant main effect of group across all days (2-way repeated-measure ANOVA).

**Results**

**Effects of AV3V Lesions on CIH Hypertension**

There were no differences between sham-lesioned and AV3V-lesioned rats observed during baseline recording of MAP, HR, or RR (please see Table S1, available in the online-only Data Supplement). During exposure to intermittent hypoxia between 8:00 AM to 4:00 PM, changes in MAP were not different between sham- and AV3V-lesioned rats (genomic DNA contamination), and 2 positive controls (for reverse transcription and PCR validation).

The quantitative PCR was run using a HotStart Taq DNA polymerase contained in the RT2 quantitative PCR master mix. Sample and master mix were loaded into the PCR array according to manufacturer instructions (SA Biosciences). Data were collected at Qiagen using a Biorad iCycler according to the following recommended protocol: (1) 95°C for 10 minutes (hot start) and (2) 40 cycles of (a) 95°C for 15 seconds; (b) 55°C for 15 seconds; and (c) 72°C for 30 seconds.

**Data Analysis and Statistics**

Effects of CIH on MAP, HR, RR, and activity during the dark phase and during the 8-hour period of CIH exposure were analyzed separately by 2-way repeated-measures ANOVA and Student-Newmann-Keuls post hoc tests (SigmaPlot version 12, Systat Software Inc, San Jose, CA). FosB/ΔFosB counts were analyzed by 1-way ANOVA with Student Newman-Keuls tests for post hoc analysis. Data from the PCR array studies were analyzed with the RT2 Profiler PCR Array Analysis Template (version 3.2, SA Biosciences). Significance was set at P<0.05. All of the values are presented as mean±SEM.

There were no differences between sham-lesioned and AV3V-lesioned rats observed during baseline recording of MAP, HR, or RR (please see Table S1, available in the online-only Data Supplement). During exposure to intermittent hypoxia between 8:00 AM to 4:00 PM, changes in MAP were not different between sham- and AV3V-lesioned rats.
Injections of the viral vectors were largely confined to the median preoptic nucleus (MnPO). A, A typical example of green fluorescent protein (GFP) labeling after 400-nL injection of adenoasociated viral vector (AAV)-GFP-ΔJunD injection targeted at the MnPO is illustrated by a Neurolucida diagram depicting the distribution of individual GFP-positive neurons in a single coronal section containing the MnPO, which is located on the midline dorsal to the third ventricle and a B corresponding photomicrograph of GFP labeling in the MnPO. Examination of periventricular lamina terminalis regions dorsal (subfornical organ; C) and ventral (organum vasculosum of the lamina terminalis; D) indicated that the injection of the viral vector did not spread to adjacent regions of the lamina terminalis or access other regions via the ventricular system. Scale bar in B is 50 μm and in C and D is 100 μm.

(Figure 1A). In sham-lesioned rats, this increase in MAP was sustained during the dark phase in normoxic conditions (Figure 1B). This sustained increase in MAP was not observed in AV3V-lesioned rats (Figure 1B). There were no differences between the groups for changes in HR recorded during CIH or the dark phase (Figure 1C and 1D) or for changes in RR either during intermittent hypoxia exposure or the dark phase (please see Figure S1 in the online-only Data supplement). The results suggest that the AV3V region is not required for the increases in MAP that occur during intermittent hypoxia exposure but may contribute to the CNS mechanisms that produce the sustained component of CIH hypertension.

**Role of MnPO ΔFosB in CIH Hypertension**

Next, we investigated the effects of dominant-negative inhibition of ΔFosB in the MnPO on the cardiovascular effects of CIH by injecting AAV vectors into this region to determine whether we would observe effects similar to the AV3V lesions. Histological examination of the injection sites showed that both viral vectors produced intense GFP labeling in the ventral and dorsal aspects of the MnPO (Figure 2A and 2B). GFP positive neurons were not observed in organum vasculosum of the lamina terminalis, subfornical organ, or in the ependymal lining of the ventricles (Figure 2C and 2D). Injections of the viral vectors had no significant effects on baseline activity or RR (please see Table S2 in the online-only Data supplement). Baseline MAP was elevated in both groups injected with AAV vectors during the light and dark phases as compared with uninjected rats, whereas HR in AAV injected rats was increased only during the light phase (please see Table S2).

During the intermittent hypoxia exposure, significant increases in MAP were observed in uninjected rats and rats injected in the MnPO with either AAV-GFP or AAV-GFP-ΔJunD as compared with normoxic controls (Figure 3A). This significant increase in MAP was sustained throughout the dark phase in the absence of hypoxia in AAV-GFP and uninjected rats exposed to CIH (Figure 3B). In contrast, this sustained increase in MAP was not evident in the rats injected in MnPO with AAV-GFP-ΔJunD (Figure 3B). During the normoxic dark phase, the MAPs of the AAV-GFP-ΔJunD-treated rats were significant lower than the other groups exposed to CIH and not different from normoxic controls (Figure 3B). All of the rats exposed to CIH demonstrated comparable increases in HR during CIH exposure independent of AAV treatment (Figure 3C). During normoxia in the dark phase, there were no differences in HR among any of the treatment groups (Figure 3D). Similar results were observed for RR and activity (please see Figure S2 in the online-only Data supplement). Thus, inhibition of MnPO ΔFosB selectively affected the sustained component of CIH hypertension without influencing changes in MAP, HR, or RR that occurred during the exposure to intermittent hypoxia. These results indicate that changes in MnPO gene expression mediated by ΔFosB are necessary for the persistent increase in MAP produced by CIH that occurs during normoxia, which may be analogous to diurnal hypertension in patients with sleep apnea.

We examined ΔFosB staining in several autonomic regulatory regions of the CNS to further determine the effects of dominant-negative inhibition of MnPO ΔFosB on the chronic transcriptional activation of these areas by CIH. Our previous work has demonstrated that CIH is associated with an increase in the numbers of ΔFosB-positive neurons in the PVN (Figure 4A), the rostral ventrolateral medulla (RVLM; Figure 4B), and the nucleus of the solitary tract (NTS; Figure 4C). The PVN and RVLM are both sympathoexcitatory regions, whereas the NTS is the primary termination point for visceral afferents, including chemoreceptors and baroreceptors. After CIH, the numbers of ΔFosB-positive cells were significantly increased in each of these areas in uninjected and AAV-GFP rats (Figure 4D). Dominant-negative inhibition of MnPO ΔFosB significantly reduced the numbers ΔFosB-positive profiles in both the PVN and the RVLM (Figure 4D), suggesting that MnPO ΔFosB is necessary for the circuit-level activation of these other regions by CIH. Furthermore, decreased translational activation of the PVN and RVLM also may contribute to the lack of a sustained CIH-mediated increase in MAP associated with MnPO ΔFosB inhibition. In the NTS, ΔFosB staining associated with CIH was not influenced by dominant-negative inhibition of ΔFosB in the MnPO (Figure 4C). In rats injected with
AAV-GFP-JunD in the MnPO, the numbers of ΔFosB-positive profiles were significantly increased compared with normoxic controls and not different from the other CIH-treated groups (Figure 4D). These findings are consistent with the observation that MnPO ΔFosB inhibition had no effect on the blood pressure or respiratory responses that occurred during CIH exposure and suggest that the MnPO does not contribute to translational activation of the NTS by CIH.

Identification of Potential Downstream Targets of MnPO ΔFosB

Based on these results, we investigated changes in MnPO gene expression associated with CIH focusing on candidate genes that could be regulated by ΔFosB and may contribute to the sustained component of CIH hypertension. Punch samples containing the MnPO were subjected to quantitative RT-PCR analysis using a customized array (SA Biosciences) that screened 86 genes with AP-1 promoter regions. Samples were collected from normoxic controls, un.injected rats exposed to CIH, and rats injected in the MnPO with either AAV-GFP or AAV-GFP-JunD before CIH. Of 86 genes analyzed, 6 were elevated after 7 days of CIH. Both angiotensin-converting enzymes (ace and ace2) were up regulated by ~50% after CIH (Figure 5A). The neuronal and endothelial isoforms of NO synthase (nos1 and nos3), mitogen activated protein kinase kinase kinase 3 (map3k3), and peroxiredoxin 2 (prdx2) also were significantly elevated after CIH (Figure 5B). The same pattern of changes in MnPO gene expression was observed in rats injected with AAV-GFP before CIH. Dominant-negative inhibition of MnPO ΔFosB significantly attenuated the CIH-mediated increases in ace, ace2, nos1, nos3, and map3k3 gene expression (Figure 5). The increase in MnPO prdx2 gene expression associated with CIH was not affected by AAV-GFP-JunD injection. For genes whose expression pattern did not change after CIH, please see Figure S3 in the online-only Data supplement.

Discussion

The main results of these experiments indicate that increased ΔFosB expression in the MnPO is necessary for sustained
hypertension associated with CIH. When rats injected in the MnPO with AAV-GFP–JunD were exposed to intermittent hypoxia during the light phase between 8:00 AM and 4:00 PM, their MAP significantly increased to levels comparable to those observed in the uninjected and AAV-GFP–injected rats exposed to CIH. After the intermittent hypoxia was discontinued each day, MAP remained significantly elevated in both uninjected and AAV-GFP–injected rats exposed to CIH. In AAV-GFP–JunD–injected rats, this component of the response to CIH was absent. During normoxia, their MAP was significantly lower than the other groups of rats that were exposed to CIH and not different from normoxic controls that were housed in similar conditions. These results indicate that changes in MnPO gene expression that are mediated by JunD are necessary for CIH to produce a hypertensive response that is maintained in the absence of intermittent hypoxia stimulation. Dominant-negative inhibition of MnPO JunD significantly reduced CIH-mediated JunD staining in both the PVN and the RVLM, which indicates that this antihypertensive effect was attributed to a decrease in centrally generated sympathetic outflow. Transcriptional activation of the NTS by CIH was not influenced by blocking MnPO JunD expression. The NTS receives direct innervation from chemoreceptor and baroreceptor afferents, which likely drive activity and JunD expression in this region during exposure to CIH independent of forebrain influences. This is consistent with the observation that rats injected in the MnPO with AAV-GFP–JunD showed a significant increase in MAP during the daily exposures to intermittent hypoxia. It also indicates that activation of the NTS by intermittent hypoxia is not sufficient to drive transcriptional activation of the PVN and RVLM or the sustained increase in MAP that is observed during the normoxic periods between intermittent hypoxia exposures.

The transcription factor JunD has been shown to contribute to neuroplasticity associated with depression, drug addiction, or other motivated behaviors. The stability of JunD appears to be a key feature allowing it to maintain plastic changes in neural function in the absence of continuous stimulation. The current findings suggest that JunD makes a similar contribution to forebrain neural networks that regulate autonomic function by mediating changes in gene expression that are necessary for sustained CIH hypertension.

We identified 5 genes in the MnPO that appear to be regulated by JunD during CIH. Expression of these genes increased after CIH exposure, and virally mediated dominant-negative inhibition of JunD prevented these changes. MAPK3 is a serine/threonine kinase that is reported to be an
Figure 5. Results of quantitative RT-PCR array analyses (SA Bioscience) using punch samples that contained the median preoptic nucleus (MnPO) from normoxic, uninjected rats (CON), chronic intermittent hypoxia (CIH)-treated uninjected rats, CIH rats injected in the MnPO region with adenoassociated viral vector (AAV)-green fluorescent protein (GFP; CIH-AAV-GFP), and CIH rats injected in the MnPO region with AAV-GFP-JunD (CIH+AAV-JunD) illustrating the effects of CIH and dominant-negative inhibition of MnPO ΔFosB. Data are expressed as normalized gene expression ratios. Data for ace and ace2 are presented in panel A and data for other genes are shown in panel B. The numbers indicated in the legend represent the number of rats used for the analysis. Extracted RNA was not pooled for quantitative RT-PCR analyses. *P<0.05 from normoxic controls (CON). Data were analyzed by 1-way ANOVA and Student-Newman-Keuls tests. ■, CON (n=5); □, CIH (n=5); △, CIH+AAV-GFP (n=5); ▲, CIH+AAV-JunD (n=4).

upstream regulator of AP-1 expression,39 angiotensin signaling,40 and several other signaling pathways31,42 in some systems. Two of these genes, ace and ace2, mediate the generation of angiotensin-related peptides that have reported to be either prohypertensive or antihypertensive.43 The expression of nos1 and nos3 was similarly affected by CIH and inhibition of MnPO ΔFosB. Decreased NOS activity in PVN is associated with elevated sympathetic outflow because of the influence of NO on local γ-aminobutyric acid release.44-46 Other studies have shown that increased NO in the lamina terminals region may be associated with increased blood pressure.47,48 In other neural systems, changes in NO activity are associated with long-term depression and long-term potentiation, and NO may participate in vasculoneuronal communication.49,50 Together these changes in MnPO gene expression could represent a substrate for cellular adaptations that are mediated by ΔFosB during CIH and support the sustained increase in blood pressure during normoxia. Additional studies will be necessary to further characterize the role of these genes and their functions in MnPO that contribute to CIH-mediated hypertension.

Previous studies have indicated that the hypertension associated with CIH is influenced by the renin-angiotensin system. CIH has been shown to increase plasma renin activity and treatment with an angiotensin receptor antagonist, delivered by daily gastric gavage, blocked the increase in blood pressure produced by CIH.51 Renal denervation attenuates both the increase in plasma renin activity and CIH-induced hypertension.52 Subsequently, it has been shown that chronic systemic angiotensin receptor blockade reduces sympathetic nerve activity in rats exposed to CIH and attenuates chemoreceptor sensitization.53 Local injection of angiotensin receptor antagonists into the PVN have been shown to attenuate CIH-induced hypertension.54 Angiotensin-related peptides that are generated by ACE2 are reported to be antihypertensive, and ACE2 overexpression has been show to influence NOS expression.55,56 Our current results suggest that central angiotensin peptides associated with the MnPO may also contribute to elevated sympathetic outflow and sustained CIH hypertension.

**Perspectives**

The sustained elevation of MAP produced by CIH, which occurs in the dark phase of the light dark cycle when the rats are normoxic, is analogous to diurnal hypertension that is associated with sleep apnea. Diurnal hypertension, that is, increased arterial pressure during waking hours, is an important pathophysiological feature of sleep apnea that is associated with an increased risk of adverse cardiovascular events. Increased MAP and sympathetic outflow in the absence of intermittent hypoxia stimulation likely requires some form of adaptation or plasticity in the neural circuits that regulate sympathetic outflow. Previous studies have demonstrated that CIH produces a sensitization of peripheral chemoreceptors that increases their activity during normoxia.56-58 Our results suggest that other CNS mechanisms also contribute specifically to the sustained component of CIH hypertension and that ΔFosB-mediated neuroplasticity in the MnPO is a critical contributing factor. A better understanding of the mechanisms that contribute to the pathogenesis and maintenance of the sustained component of the CIH hypertensive response may provide us with a better understanding of diurnal hypertension in sleep apnea patients.

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Disclosures

None.

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**Novelty and Significance**

**What Is New?**

- Our study indicates that the CNS contributes to sustained hypertension associated with intermittent hypoxia.
- This contribution involves activity-dependent changes in gene expression that may change the way the neurons function as part of a network that controls the sympathetic nervous system.
- Preventing these changes in gene expression selectively reduced the hypertension that is maintained in the absence of hypoxia, which is similar to diurnal hypertension in patients with sleep apnea.

**What Is Relevant?**

- The antihypertensive response occurred only during periods of the day when the animals were not being exposed to the hypoxia.
- These findings could provide new insight to how hypertension develops in association with sleep apnea.

**Summary**

Our results show that FosB-mediated changes in gene expression contribute to hypertension in an animal model of hypoxia related to sleep apnea. We identified 5 target genes and specific regions of the brain that may be responsible for these effects.
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An essential role for ΔFosB in the median preoptic nucleus in the sustained hypertensive effects of intermittent hypoxia.

Abbreviated title: MnPO ΔFosB contributes to IH-induced hypertension

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Supplementary Table S1: Average baseline recordings obtained from 0800-1600 h during the light phase exposure to intermittent hypoxia (IH) and during the dark phase (DK) for each group.

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Supplementary Table S2: Average baseline recordings obtained from 0800-1600 h during the light phase exposure to intermittent hypoxia (IH) and during the dark phase (DK) for each group.

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* P < 0.05 from CON. † P < 0.05 from CIH. One-way ANOVA and Student-Newman-Keuls tests.
Supplementary Figure S1: Changes in respiratory frequency during intermittent hypoxia exposure (left) and during the normoxic dark phase (right) in sham lesioned and AV3V lesioned rats. There were no differences between the groups for either time period.
Supplementary Figure S2: (a) During intermittent hypoxia exposure uninjected rats exposed to CIH demonstrated changes in respiratory rate significantly higher than normoxic controls while CIH rats injected with the two viral vectors demonstrated increases in respiratory rate that were greater than the other two group but not different from each other. (b) There were no significant differences among the 4 groups for respiratory rate recorded during normoxia during the dark phase. (c & d) There were no significant differences among groups for activity during intermittent hypoxia exposure or the normoxic dark phase. Data were analyzed by two-way repeated measures ANOVA. ** is different from control. * is different from control and CIH.
Supplementary Figure S3: Genes with consensus AP-1 regulatory domains that were not affected by CIH. MnPO samples were obtained from normoxic controls and uninjected rats exposed to CIH for 7 days. Data are expressed as fold differences. Samples from individual rats were analyzed separately. n = 5 per group. * indicate genes with an average Ct > 30.

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