Chronic intermittent hypoxia increases sympathetic control of blood pressure: role of neuronal activity in the hypothalamic paraventricular nucleus

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Sharpe AL, Calderon AS, Andrade MA, Cunningham JT, Mifflin SW, Toney GM. Chronic intermittent hypoxia increases sympathetic control of blood pressure: role of neuronal activity in the hypothalamic paraventricular nucleus. Am J Physiol Heart Circ Physiol 305: H1772–H1780, 2013. First published October 4, 2013; doi:10.1152/ajpheart.00592.2013.—Like humans with sleep apnea, rats exposed to chronic intermittent hypoxia (CIH) experience arterial hypoxemias and develop hypertension characterized by exaggerated sympathetic nerve activity (SNA). To gain insights into the poorly understood mechanisms that initiate sleep apnea/CIH-associated hypertension, experiments were performed in rats exposed to CIH for only 7 days. Compared with sham-treated normoxic control rats, CIH-exposed rats (n = 8 rats/group) had significantly increased hematocrit (P < 0.001) and mean arterial pressure (MAP; P < 0.05). Blockade of ganglionic transmission caused a significantly (P < 0.05) greater reduction of MAP in rats exposed to CIH than control rats (n = 8 rats/group), indicating a greater contribution of SNA in the support of MAP even at this early stage of CIH hypertension. Chemical inhibition of neuronal discharge in the hypothalamic paraventricular nucleus (PVN) (100 pmol muscimol) had no effect on renal SNA but reduced lumbar SNA (P < 0.005) and MAP (P < 0.05) in CIH-exposed rats (n = 8) than control rats (n = 7), indicating that CIH increased the contribution of PVN neuronal activity in the support of lumbar SNA and MAP. Because CIH activates brain regions controlling body fluid homeostasis, the effects of internal carotid artery injection of hypertonic saline were tested and determined to increase lumbar SNA more (P < 0.05) in CIH-exposed rats than in control rats (n = 9 rats/group). We conclude that neurogenic mechanisms are activated early in the development of CIH hypertension such that elevated MAP relies on increased sympathetic tonus and ongoing PVN neuronal activity. The increased sensitivity of Na+/H+ exchange in CIH-exposed rats suggests that early neuroadaptive responses among body fluid regulatory neurons could contribute to the initiation of CIH hypertension.

body fluid balance; sympathetic nerve activity; sleep apnea; hypertension; hyperosmolality

SLEEP APNEA (SA) is a common medical condition often accompanied by arterial hypertension (28, 63, 65). Exposure of animals to chronic intermittent hypoxia (CIH) is a frequently used experimental model that mimics the repetitive arterial hypoxemias experienced during SA (12, 13, 50). Most CIH protocols expose rats or mice to repetitive bouts of hypoxia during their nocturnal period on consecutive days. Although protocols vary in terms of the severity and timing of hypoxic periods, a consistent finding across laboratories is that arterial hypertension develops rapidly and persists during the hours of the day that animals breathe normoxic air (2, 13, 14, 32). The latter feature is important because it mimics hypertension in patients with SA (43–45, 62), but a detailed understanding of the mechanisms that initiate the hypertensive response in humans is lacking.

Whereas maintenance of CIH-induced hypertension is generally agreed to depend on increased systemic vascular resistance, the underlying mechanisms are complex and involve changes in vascular reactivity (1, 22–24, 47) as well as chronically elevated sympathetic nerve activity (SNA) (16, 32, 60). The latter has been strongly linked to sensitization of the arterial chemoreflex (15, 32, 45, 50, 67), which results in tonic activation of sympathetic outflow even at normoxic arterial PO2. In previous studies (8, 20, 27, 35, 53, 67), we sought to identify the early neural adaptations that trigger sustained CIH-induced sympathetic hyperactivity and hypertension using a model of CIH in which rats were exposed to CIH for just 7 days, with hypertension developing over the first ~3 days and remaining at a stable level thereafter. By day 7 of CIH exposure, rats exhibit an augmented hypothalamic-pituitary-adrenal axis response to restraint stress and increased expression of c-Fos in the locus coeruleus (35), effects that are consistent with another model of CIH (68). Our CIH-exposed rats also exhibit increased expression of FosB/ΔFosB transcription factors that index chronic neuronal activation. Robust FosB/ΔFosB expression occurs in body fluid regulatory regions of the forebrain lamina terminalis (8, 27), such as the median preoptic nucleus (MnPO). Expression is also observed in downstream sympathetic regulatory cell groups, including neurons in the hypothalamic paraventricular nucleus (PVN) and rostral ventrolateral medulla (8, 27). Importantly, full expression of hypertension in our 7-day CIH model critically depends on activator protein-1 transcriptional regulation of MnPO neurons by FosB/ΔFosB (8).

These and other observations (7, 9, 40, 41, 46, 70) indicate that exaggerated SNA and the neurogenic component of CIH-induced hypertension are complex and might not arise solely from the sensitization of arterial chemoreceptors. In the present study, we sought to determine the early contribution of forebrain/hypothalamic neural mechanisms in hypertension induced by 7 days of CIH. We first established that interruption of ongoing autonomic activity by ganglionic blockade caused a greater reduction of mean arterial pressure (MAP) in CIH-
exposed rats than in normoxic control rats, supporting the view that heightened sympathetic tonus contributes to support of MAP even at this early stage of CIH hypertension. Next, we determined that acute chemical inhibition of PVN neuronal activity caused greater reductions of lumbar SNA (LSNA) and MAP in CIH-exposed rats than in normoxic control rats. Acute hypertonic saline stimulation of the forebrain revealed that CIH-exposed rats had exaggerated lumbar sympathoexcitatory and pressor responses compared with control rats. We conclude that 7 days of CIH exposure is sufficient to induce hypertension mediated, at least in part, by neurogenic mechanisms involving increased sympathetic tonus and enhanced reliance on ongoing PVN neuronal activity. Neuroadaptive responses to CIH further lead to heightened sensitivity of Na+/osmosensitive regions of the brain that regulate sympathetic outflow.

**METHODS**

**Animals**

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 300–325 g were housed in a temperature-controlled room (22–23°C) with a 14:10-h light-dark cycle (lights on at 07:00 hours). Standard rat chow (LM-485, Harlan Teklad) and tap water were available ad libitum. All protocols and procedures were performed in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the University of Texas Health Science Center (San Antonio, TX).

**Telemetric Recordings**

Radio telemetry (Data Sciences, St. Paul, MN) was used to continuously record arterial blood pressure (ABP) and heart rate (HR) as previously described (5, 8, 20, 27, 35, 53). Transmitters were implanted under isoflurane (2–3% in O2) anesthesia, and a model PA-C40 transmitter was inserted −10–15 mm into the abdominal aorta. Transmitter catheters were secured with tissue adhesive (Vetbond, Butler Schein, Dublin, OH), and the transmitter housing was sutured to the abdominal wall. Each rat was allowed to recover from surgery for at least 7 days before the CIH protocol began.

**Exposure to Intermittent Hypoxia**

Rats in their home cages were placed singly inside custom-built Plexiglas chambers 5–7 days before the 7-day CIH protocol began, as previously described (8, 20, 27, 35, 53). Briefly, the percentage of O2 in each chamber was monitored and regulated by two timer-controlled valves: one valve supplying pressurized room air and the other valve supplying 100% N2. Flow control valves allowed the delivery of each gas to be independently adjusted. Each rat was exposed to CIH for 8 h/day from 08:00 to 16:00 hours for 7 consecutive days. During CIH exposure, chamber O2 was repetitively cycled between 21% and 10% with a period of 6 min (10 cycles/h). Control rats underwent sham treatment, which was identical except that both chamber valves supplied room air (21% O2) so that rats remained normoxic throughout the protocol. Telemetry signals (ABP and HR) were acquired for 5–7 days before (baseline) and throughout the 7-day sham/CIH protocol. All experiments were performed 18–20 h after completion of the last CIH exposure.

**Ganglionic Blockade in Conscious Rats**

During surgical implantation of telemetry transmitters, cohorts of sham-treated (n = 8) and CIH-exposed (n = 8) rats were also instrumented with an indwelling catheter (Tygon tubing) in a femoral vein, which was tunneled subcutaneously and exited between the scapulae. After rats had recovered as described above, stable baseline telemetric recordings of ABP and HR were acquired for 5–10 min, after which hexamethonium (20 mg/kg iv) was given to block neurotransmission through autonomic ganglia. The effects on mean ABP (MAP) and HR were determined 5 min later. For these recordings, telemetric signals were passed through a model R11CPA analog output unit (Data Sciences) and were acquired using a Micro1401 analog-to-digital converter and Spike2 software (version 6, Cambridge Electronic Design, Cambridge, UK).

**Acute Experiments in Anesthetized Rats**

Rats were anesthetized initially with isoflurane (3% in 100% O2), and catheters (PE-50 tubing) were implanted into a femoral vein for drug delivery and blood sample collection. A femoral artery catheter was implanted for direct recording of ABP. HR was determined from a lead I ECG. Rats were then transferred to urethane/o-chloralose (800/80 mg/kg iv) anesthesia. After tracheal cannulation, rats were ventilated with O2-enriched room air and paralyzed with gallamine triethiodide (25 mg/kg iv bolus and 5 mg·kg⁻¹·h⁻¹ iv infusion). End-tidal CO2 was maintained between 4% and 5% by adjusting the ventilation rate (75–85 breaths/min) and/or tidal volume (2.0–2.5 ml). Supplements of anesthetic (10% of the initial dose, intravenously) were given as needed to maintain the stability of baseline MAP and HR. Normal body temperature (37 ± 1°C) was maintained with a water circulating pad.

**Recordings of SNA.** Through a dorsal flank incision, a renal nerve bundle and a segment of the lumbar sympathetic chain were isolated. Each was placed on a bipolar stainless steel wire (outer diameter: 0.005 in., A-M Systems, Everett, WA) electrode and insulated from body fluid with silicone-based epoxy (Kwik-Sil, World Precision Instruments, Microcast, Arrowhead Forensics). Signals were amplified (20–50,000×), band-pass filtered (30–1,000 Hz), and digitalized (1,500 Hz). Recorded signals were full-wave rectified and integrated (time constant: 3 s) using Spike2 software (version 6, Cambridge Electronic Design,). Rats were allowed to stabilize after surgery for 30–60 min before acute experimental protocols began.

**PVN microinjections.** Sham (n = 7) and CIH-exposed (n = 8) rats were placed in a stereotaxic frame and underwent a craniotomy to access the PVN. A single-barreled glass micropipette (tip outer diameter: 20–30 μm, World Precision Instruments, Microcast, Arrowhead Forensics) was vertically lowered into PVN at the following coordinates: 1.9 mm caudal to the bregma, 0.4 mm lateral to the midline, and 7.6–7.8 ventral to the dura. To inhibit neuronal activity, the GABA-A receptor agonist muscimol (100 pmol) was injected bilaterally into the PVN over a period of ~30 s in a volume of 50 nl/side. Identical procedures were used for control injections of artificial cerebrospinal fluid vehicle, which were performed in the same rats ~30 min before injections of muscimol.

**Histology.** Injection sites were marked by including 0.2% rhodamine microspheres (Lumafluor, Naples, FL) in the muscimol injectate. Upon completion of each experiment, rats were euthanized (100 mg/kg iv pentobarbital), and brains were removed and postfixed in 4% paraformaldehyde in PBS for 3–5 days at 4°C. Sections (50 μm) were cut through the PVN with a cryostat, and the distribution of microinjected fluorescent microspheres was determined under a fluorescence microscope fitted with the appropriate filters (wavelength: 530/590 nm). The fluorescence distribution for each rat was determined as previously described (5, 53, 55, 57). Briefly, digital images of PVN sections were taken with a SPOT camera (Diagnostic Imaging), and the outermost fluorescence boundary was traced. Traced areas were then mapped onto the appropriate rostrocaudal plates of the PVN. Traced outlines from similar rostrocaudal levels of the PVN were overlaid, and a final trace was made of the largest distribution of fluorescence for each group.
**Table 1. Effects of sham and CIH exposure on resting MAP, HR, and Hct and effects of ganglionic blockade**

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<th>Baseline</th>
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<td>MAP, mmHg</td>
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<td>Sham group</td>
<td>50 ± 1</td>
<td>98 ± 4</td>
<td>110 ± 5</td>
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<td>CIH group</td>
<td>62 ± 2**</td>
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Values are means ± SE; n = 8 rats/group. CIH, chronic intermittent hypoxia; MAP, mean arterial pressure; HR, heart rate; Hct, hematocrit. *P < 0.05 vs. the sham group; **P < 0.001 vs. baseline (MAP) and the sham group (Hct); †P < 0.05 vs. the sham group.

**Internal Carotid Artery Injections of Hypertonic Saline**

In separate groups of sham (n = 9) and CIH-exposed (n = 9) rats, osmotic-sensitive regions of the forebrain were stimulated as previously described (6, 54, 55, 58, 59). Briefly, warmed (37°C) aqueous solutions of isotonic (290 mosM) and hypertonic (870 mosM) saline (NaCl) were delivered in a volume of 100 μl over 10–15 s through a flame-pulled fine-tipped catheter (PE-50 tubing) inserted into the internal carotid artery (ICA). Catheters were advanced well beyond the carotid sinus, and a ligature was placed upstream to prevent sinus pressurization and arterial baroreceptor activation during ICA injections.

**Data Analysis and Statistics**

Baseline MAP and HR obtained from radiotelemetry signals were determined as the mean of 24-h averages for 5 consecutive days before initiation of the sham/CIH protocol. Effects of ganglionic blockade on MAP in conscious sham and CIH-exposed rats were determined as the difference between a 5-min baseline and the value 5 min after hexamethonium. For PVN microinjection experiments, values of recorded variables at baseline and 30 min after injections of muscimol were taken as 60-s averages. For ICA injections of isotonic and hypertonic saline, responses of recorded variables were determined as the difference between a 60-s average at baseline and the peak response that occurred within 3 min of each injection. In all cases, renal SNA (RSNA) and LSNA responses are expressed as percentages of baseline after the subtraction of electrical noise, which was determined for each nerve recording as the signal remaining 5 min after ganglionic blockade with hexamethonium. Effects of PVN muscimol and ICA injections of isotonic and hypertonic saline were separately compared across groups of sham and CIH-exposed rats using two-way ANOVA. Independent t-tests with the Bonferroni correction were performed to compare means from control and CIH-exposed groups. P values of <0.05 were considered significant. Values in the text and figures are expressed as means ± SE.

**RESULTS**

**Short-Term CIH Induces Neurogenic Hypertension**

Seven days of CIH exposure significantly increased hematocrit (P < 0.001) and MAP (P < 0.05) relative to sham treatment (n = 8 rats/group; Table 1). HR was unaffected, as previously reported (20, 27, 35, 53, 64). In addition, blockade of ganglionic transmission with hexamethonium in conscious rats significantly reduced MAP in both groups (P < 0.001; Table 1). The average fall of MAP, however, was significantly greater in the CIH-exposed group compared with the sham control group (P < 0.05), such that MAP values after hexamethonium were no longer different across groups. These data indicate that ongoing SNA plays a greater role in supporting elevated MAP in CIH-exposed rats than in normoxic control rats.

**Time Course of CIH-Induced Hypertension**

Figure 1 shows values of MAP (A) and HR (B) in sham-treated (n = 7) and CIH-exposed (n = 8) rats. Whereas HR was not different across groups at baseline or during sham/CIH exposure, MAP increased during the first 2 days of CIH and remained significantly elevated compared with the sham group throughout most of the remaining 5 days of the protocol (P < 0.05–0.01).

![Fig. 1. Effects of sham treatment and chronic intermittent hypoxia (CIH) exposure on mean arterial pressure (MAP; in mmHg) and heart rate (HR; in beats/min [BPM]) in conscious rats. Baseline values were recorded for 5 days before 7-day sham and CIH protocols were initiated. A: values of MAP in each group were similar at baseline but increased during the first 2 days in CIH-exposed rats (n = 8) to become significantly greater than sham-treated rats (n = 7) during most of the remaining protocol. B: values of HR were similar across groups at baseline and throughout the sham and CIH protocols. Note: data represent average values recorded from 08:00 to 16:00 hours each day, i.e., when rats in the CIH-exposed group experienced bouts of intermittent hypoxia. *P < 0.05 and †P < 0.01 vs. the sham group.](http://ajpheart.physiology.org/)

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Effects of PVN Muscimol

Figure 2A shows representative responses of a sham (left) and CIH-exposed (right) rat to bilateral PVN microinjection of the GABA-A receptor agonist muscimol. Whereas the sham-treated rat showed little response in terms of LSNA (top), RSNA (middle), or ABP (bottom), PVN muscimol caused pronounced reductions of LSNA and ABP in the CIH-exposed rat but, again, had little effect on RSNA. Summary data shown in Fig. 2B demonstrate that, whereas PVN muscimol significantly reduced MAP in sham (P < 0.05) and CIH-exposed (P < 0.001) groups, the average fall was significantly larger in the CIH-exposed group (P < 0.05). Interestingly, PVN muscimol did not change ongoing RSNA in either group but caused a significantly greater reduction of LSNA in the CIH-exposed group than in the sham group (P < 0.005). Collectively, data indicate that the greater fall of MAP in CIH-exposed rats likely reflects, at least in part, the effect of muscimol to reduce PVN-mediated support of LSNA.

Histological analysis of microinjection sites revealed that muscimol was consistently delivered into the center of the PVN bilaterally. Figure 3A shows a photomicrograph of a mid-PVN coronal section and shows fluorescence from injected microspheres distributed over the dorsal and central aspects of the PVN. Figure 3B shows the distribution of fluorescence for all rats in the sham (left) and CIH-exposed (right) groups. Note that injected microspheres covered the majority of the PVN in both groups (Fig. 3B, top left and top right) except for the most lateral portions near the caudal end of the nucleus (Fig. 3B, bottom left and bottom right). It should be noted that the distribution of microspheres likely underestimates the distribution of muscimol due to the relatively restricted diffusion of microspheres.

Effects of ICA Injection of Hypertonic Saline

Figure 4A shows representative effects of ICA injections (arrow) of isotonic (top) and hypertonic (bottom) saline in a sham-treated (left) and CIH-exposed (right) rat. As expected, LSNA (top trace), RSNA (middle trace), and ABP (bottom trace) were unaffected by isotonic saline, but hypertonic saline promptly increased all recorded variables in both the sham and CIH-exposed rat (Fig. 4A). However, the increase of LSNA was larger in the CIH-exposed rat. Summary data shown in Fig. 4B demonstrate that isotonic saline had no effect on recorded variables in either the sham (n = 9) or CIH-exposed (n = 9) groups, but hypertonic saline significantly increased all recorded variables (P < 0.05). Of note, the average increase of LSNA by ICA-injected hypertonic saline was significantly larger in the CIH-exposed group than in the sham group (P < 0.05).

DISCUSSION

In this study, rats exposed to 7 days of CIH developed a stable level of hypertension, as previously reported (8, 20, 27, 67). Even at this early stage of hypertension, ganglionic blockade revealed that ongoing SNA significantly contributed to the maintenance of elevated MAP. Thus, in keeping with other studies of CIH, our protocol induced hypertension with a significant neurogenic component (53, 71). Here, we report that the maintenance of both sympathetic drive and hypertension was reliant on ongoing activity of neurons in the hypo-
vascular reactivity changes clearly play an important role (1, 22–24, 42, 47–49, 60), as does sustained tonic hyperactivity and acute hyperexcitability of neurons comprising sympathetic regulatory pathways.

It is generally agreed that the maintenance of hypertension induced by CIH depends on increased systemic vascular resistance. Indeed, vascular reactivity changes clearly play an important role (1, 22–24, 42, 47–49, 60), as does sustained activation of sympathetic outflow (16, 32, 60). In the present study, blockade of ganglionic transmission caused a greater fall of MAP in CIH-exposed rats than in normoxic control rats. No significant effect on HR was observed in either group. The persistence of these vascular adaptations during ganglionic blockade would be predicted to maintain a higher level of MAP in the CIH-exposed group than in the normoxic control group. It is presently unclear why this was not observed, but a possible explanation is that intrinsic vasoactive mechanisms in CIH-exposed rats do not effectively maintain higher MAP when peripheral vessels are devoid of convergent adrenoceptor-mediated neurogenic vasomotor tone. Evidence in the literature supports this possibility (29, 51, 56, 61, 66). It could also be that the maintenance of higher MAP during ganglionic blockade requires structural remodeling of the vasculature, which has been documented to occur after 14 days of CIH (3) but might not have occurred in the present study after only 7 days of exposure. Consistent with this possibility, a previous study (9) has reported that MAP during ganglionic blockade was greater in rats exposed to CIH for 35 days compared with normoxic control rats.

The persistence of sympathoexcitation during normoxic periods of the day is a hallmark of obstructive SA and CIH (28, 43, 45, 63). This has been linked to sensitization of the arterial chemoreflex (15, 32, 45, 50, 67), which results from a complex mixture of mechanisms ranging from increased O2 sensitivity of carotid body chemoreceptors (11, 21, 30, 31, 50) to enhanced sympathoexcitatory neurotransmission through the brain stem arterial chemoreflex arc (10, 18, 38, 50, 67). However, emerging evidence indicates that persistent neurogenic CIH hypertension involves additional adaptive mechanisms that contribute to heightened sympathetic drive. For example, CIH hypertension involves chronic activation of body fluid and autonomic regulatory regions of the forebrain lamina terminalis and brain stem (10, 26, 27, 36, 70). Among lamina terminalis regions activated by CIH, the MnPO appears to be particularly important. Not only do MnPO neurons heavily innervate the downstream hypothalamic PVN (39, 58, 59), but virus-mediated expression of a mutant JunD construct that interferes with FosB/ΔFosB transcriptional regulation in MnPO neurons largely prevents the sustained hypertension during normoxia resulting from 7 days of CIH (8). The latter is consistent with results from previous reports (7, 9, 25, 27, 53) and the present study, which indicate that CIH tonically activates the downstream PVN.

These and other (40, 41, 46, 70) observations indicate that the neurogenic component of CIH-induced hypertension likely develops rapidly and involves more than sensitization of the arterial chemoreflex. Additional support for this possibility has been provided recently by da Silva et al. (9), who reported that chronic blockade of ANG II type 1 receptors in the hypothalamic PVN significantly attenuated the development of CIH hypertension. This is consistent with evidence showing that CIH increases activity of the renin-angiotensin system (17) and that systemic blockade of ANG II type 1 receptors reduces CIH hypertension (37, 38). Still further evidence for the involvement of neuroadaptive responses in the mediation of CIH-induced hypertension was provided recently by Coleman et al. (7), who reported that mice exposed to CIH for 14 or 35 days showed marked internalization of the required N-methyl-D-aspartate (NMDA) receptor subunit NR1 in the PVN. Of note, the reduction of NMDA current among PVN neurons observed by Coleman et al. was associated with reduced production of neuroinhibitory nitric oxide (NO), but the latter was not ob-

Fig. 3. Histological verification of PVN injection sites. A: representative photomicrograph of a coronal section (50 μm) through the PVN showing the distribution of rhodamine-containing microspheres cojected with muscimol in a sham-treated rat. 3V, third ventricle. B: schematic drawings of PVN microinjection sites in sham (left; n = 7) and CIH-exposed (right; n = 8) rats. The shaded regions represent the farthest distribution of microspheres among all rats comprising each group (not a typical example from a single rat). Stereotaxic coordinates between images are referenced to the bregma.

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served until 35 days of CIH exposure. Studies are clearly warranted to identify the mechanisms that underlie these adaptive responses, but available time course data suggest that significant blunting of NO-mediated inhibition is unlikely to have occurred in the PVN after only 7 days of CIH.

Another observation that is presently unexplained is that CIH increased the role of PVN neuronal activity in support of LSNA but did not enhance PVN-mediated support of RSNA. This raises the possibility that greater than 7 days of CIH exposure may be required to fully recruit the sympathetic regulatory pathways of the PVN that supply the kidneys. Alternatively, it may be that the PVN is organized to more strongly regulate LSNA than RSNA in the setting of CIH exposure. Such an organization might not uniquely impact the control of SNA in response to CIH since we (57) have previously reported that PVN inhibition in water-deprived rats reduced LSNA significantly more than RSNA. Collectively, evidence seems to suggest that a variety of homeostatic challenges might be capable of shifting PVN control to favor LSNA over RSNA. Clearly, neuroanatomic and functional studies to determine the extent to which PVN neurons differentially innervate/functionally impact different end organs/tissues is beyond the scope of the present study. However, such studies could shed important light on the pathophysiologic consequences of acute or chronic PVN activation.

In this study, we observed that ICA injection of hypertonic saline elicited an exaggerated increase of LSNA in CIH-exposed rats compared with normoxic control rats. According to previous studies (54, 55), hypertonic saline increases SNA and MAP through a mechanism that depends largely on neurons of the forebrain organum vasculosum laminae terminalis, which an anatomic study (19) has shown to send mostly glutamatergic projections to downstream sympathetic control regions of the PVN. It should be acknowledged that the exaggerated LSNA response to hypertonicity observed in CIH-exposed rats could have arisen from the recruitment of PVN-independent sympathoexcitatory mechanisms or neural pathways. To the extent that PVN neurons do mediate exaggerated LSNA responses to hypertonicity, then the CIH-induced internalization of NMDA NR1 receptor subunits report by Coleman et al. (7) would be predicted to cause a blunted, not exaggerated, sympathoexcitatory response. However, after 35 days of CIH, reduced membrane localization of NMDA receptors was associated with blunted NMDA-stimulated production of NO. Given that NO in the PVN is known to tonically facilitate GABA-A receptor-mediated neuronal inhibition (4, 33, 34, 52), it might be that concurrent loss of functional NMDA receptors and NO-mediated inhibition by CIH could result, on balance, in PVN neurons having greater than normal responsiveness to glutamatergic activation by forebrain inputs. This
remains to be experimentally tested. As noted above, available time course data suggest that blunted NO inhibition may be unlikely to occur after only 7 days of CIH.

In the present study, the greater peak increase of LSNA elicited by ICA injection of hypertonic saline in CIH-exposed rats was not accompanied by a larger increase of MAP. A possible explanation is that enhancement of the LSNA response in CIH-exposed rats was not of sufficient magnitude to result in a greater increase of blood pressure. An alternative explanation was recently provided by Silva and Schreihofer (56), who reported that sympathoexcitatory responses to a variety of acute stimuli were exaggerated in rats exposed to CIH for 14 days. Pressor responses to the same stimuli were normal, and this was linked to reduced vascular reactivity to \( \alpha \)-adrenoceptor activation. If reduced vascular reactivity is present after only 7 days of CIH, then a similar mechanism could explain the present findings.

In summary, our results indicate that even short-term exposure to CIH induces sustained hypertension driven by heightened sympathetic vasomotor tone and by ongoing activity of neurons in the hypothalamic PVN. Additional studies are needed to determine the molecular mechanisms of CIH-induced hypertension and to identify which neuroanatomic and neurochemical classes of PVN neurons are the substrate(s) for CIH-induced sympathetic drive. This notwithstanding, the present study reveals that hypertension resulting from 7 days of CIH exposure has a significant neurogenic component. Of note, the exaggerated sympathetic “tone” emanating from PVN is accompanied by an exaggerated lumbar sympathoexcitatory response to acute hyperosmotic activation of the forebrain. This suggests that CIH induces relatively rapid adaptive responses that drive tonic activity of sympathetic-regulatory neurons of the PVN and possibly increases their acute excitability. Further investigation is needed to identify neuroadaptations to CIH that occur among circuit elements lying proximal and distal to the PVN and to reveal their respective roles in mediating the early neurogenic component of CIH-induced hypertension.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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