Oxytocin neuron activation prevents hypertension that occurs with chronic intermittent hypoxia/hypercapnia in rats

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Oxytocin neuron activation prevents hypertension that occurs with chronic intermittent hypoxia/hypercapnia in rats. Am J Physiol Heart Circ Physiol 310: H1549–H1557, 2016. First published March 25, 2016; doi:10.1152/ajpheart.00808.2015.—Hypertension is a common outcome associated with obstructive sleep apnea (OSA), a prevalent yet poorly treated cardiovascular disease. Recent studies showed oxytocin (OXT), released from hypothalamic paraventricular nucleus (PVN) neurons, activates cardiac vagal neurons in the dorsal motor nucleus of the vagus (DMNX) and may blunt cardiovascular responses to stress. This study tests whether the release of OXT from PVN fibers in the DMNX is diminished with chronic intermittent hypoxia-hypercapnia (CIH/H) exposure, an animal model of OSA, and whether activation of PVN OXT neurons restores OXT release in the DMNX and prevents the hypertensive response from CIH/H. To assess OXT release from PVN fibers, Chinese hamster ovarian (CHO) cells were engineered to be highly sensitive to OXT by stable expression of the human recombinant OXT receptor and the calcium indicator R-GECO1. PVN fibers in the DMNX were selectively photoactivated in vitro by expression of channelrhodopsin. The release of OXT onto CHO cells in the DMNX was blunted in rats exposed to 21 days of CIH/H. Chronic activation of PVN OXT neurons in vivo, using designer receptors exclusively activated by designer drugs, restored the release of OXT onto CHO cells in the DMNX. Chronic PVN OXT neuron activation in vivo also prevented the hypertension that occurred in conscious unrestrained telemetry-equipped sham rats exposed to 3 wk of CIH/H. These results demonstrate that chronic activation of OXT neurons restores the release of OXT from PVN fibers in the DMNX and prevents the hypertension that occurs with 3 wk of CIH/H exposure.

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facilitation in the excitatory monosynaptic pathway from the PVN to CVNs (10). However, these studies did not test whether OXT release is blunted with CIH/H and, importantly, whether there is any in vivo functional consequences of restoring OXT neuron activity if it is reduced with CIH/H (10).

This study tests the hypothesis that the release of OXT from PVN fibers in the DMNX is blunted following 3 wk of CIH/H. Furthermore, this study tests whether chronic activation of PVN OXT neurons in vivo restores the release of OXT in the DMNX that is blunted by CIH/H and whether selectively silencing PVN OXT neuron expression frames (28, 29). Sham animals received injections of the Cre-expressing virus but without the Cre-expressing virus. OXT receptors, as well as the red fluorescent calcium indicator, were expressed in PVN OXT neurons in conscious telemetry-equipped animals mitigates the development of hypertension that occurs with CIH/H.

METHODS

Ethical approval. All animal procedures carried out were in accordance with The George Washington University Institutional Guidelines and in compliance with the recommendations of the panel of Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication Guide for the Care and Use of Laboratory Animals (revised 2011). All procedures were approved by the George Washington University Institutional Animal Care and Use Committee. The minimal number of animals was used, and care was taken to reduce any possible discomfort.

Viral vectors, plasmids, and promoter constructs. Lentiviral plasmids pLenti-Syn-hChR2(H134R)-EYFP-WPRE, packaging plasmid pCMV-ΔR8.74, and envelope plasmid pMD2.G were all kindly donated by K. Deisseroth (Stanford University, Stanford, CA). The pLenti-Syn-hChR2(H134R)-EYFP-WPRE viral vector was produced according to customary protocols, as described previously (28, 29).

A rat minimal OXT promoter element from −530 bp to +33 bp relative to the origin of transcription of the OXT gene (UCSC genome browser on rat Nov. 2004 assembly; chr3:118,193,690 to 118,194,252) was synthesized de novo and flanked by multiple cloning sites (Genscript) (28, 29). The rAAV1-OXT-Cre was produced using the OXT promoter fragment pFB-AAV-OXT. The Cre promoter was created by cloning the OXT promoter into pVSV2 by excising the OXT promoter/pUC57 with XbaI (5') and AgeI (3') and cloning it into pVSV2 cut with SpeI (5') and AgeI (3'). Then Cre was added by cutting Cre out of pBS185 with Xhol (5') and MluI-blunt (3') and moving it into pFB-AAV-OXT cut with Xhol (5') and Asp718-blunt (3'). To achieve robust and highly selective expression of designer receptors exclusively activated by designer drugs (DREADDs) in PVN OXT neurons, the reporter viral vector AAV2-hSyn-DIO-hmDiD(G4)3-mCherry (UNC, Gene Therapy Center, Vector Core Services) was coinjected with AAV-OXT-Cre. Expression of these Cre-dependent vectors was only initiated in neurons selectively expressing Cre, as they contain silencing double-blunted inverse open reading frames (28, 29). Sham animals received injections of the DREADDs virus but without the Cre-expressing virus. OXT receptors, as well as the red fluorescent calcium indicator, were expressed in CHO cells as previously described (29). CHO cells were transfected with pCDNA3.1+ containing human OXT receptor cloned in at EcoRI (5') and Xhol (3') (plasmid obtained from Missouri S&T CDNA Resource Center) using Lipofectamine, and stable overexpression was achieved by geneticin (500 μg/ml) selection. OXT receptor-expressing CHO cells were then plated and transiently transfected to also express the red fluorescent genetically encoded calcium indicator (R-GECO1; plasmid kindly donated by Robert Campbell, University of Alberta, Canada; Addgene plasmid 32444) with Fugene 6.

Stereotactic injections. Stereotactic injections into the PVN were performed as previously described (10, 28, 29). Neonatal Sprague-Dawley male rats (postnatal day 4 or 5, P4 and P5) were anesthetized by hypothermia and mounted in a stereotactic apparatus with a neonatal adapter (Stoelting). A midline incision exposed the skull, and a small burr hole was made to position a pulled calibrated pipette (VWR) with a thin tip (inner diameter <30 μm) containing 75–100 nl of pLenti-Syn-hChR2(H134R)-EYFP-WPRE and/or 20–30 nl of viral vectors rAAV1-OXT-Cre and AAV2-hSyn-DIO-hmDiD(G4)3-mCherry at a 1:2 ratio at the followingcoordinates: 1.50–1.95 mm posterior (depending on the distance between bregma and lambda) and 0.3 mm lateral relative to the lambda. The pipette tip was lowered 5.15–5.25 mm from the dorsal surface of the brain, and the entire volume of viral vector was injected at a visually monitored rate of 60 nl/min. The pipette was left in place for 10 min before slow retraction. The incision was closed using surgical glue, antibiotic ointment was applied to closed incisions, and animals were warmed up on a heating pad before being returned to their nest. The stereotactic PVN coordinates were verified after each experiment for accuracy of the injection sites, and animals with expression of ChR2 or DREADDs outside of the PVN were eliminated from the study.

Air or CIH/H. As previously described (10, 11), male Sprague-Dawley rats that previously underwent stereotaxic injections and, if necessary, telemetry implants were transferred to commercial atmospheric-controlled chambers in their cages. Using a commercial chamber with computer-controlled atmospheric gas control (Biospherix), rats were kept in cages with normal bedding and unrestricted food and water during CIH/H. Bioactive gas sensors in the chambers monitored oxygen and CO2 concentrations and exchanged these gases within the chamber until the defined set points were reached. CIH/H exposure was performed by cycling between room air (21% O2-79% N2) and mild H/H (6% O2-5% CO2-89% N2) 10 times each hour, 8 h each day, for 21 days. Each CIH/H cycle had four phases each of 90 s: 1) room air was changed to H/H, 2) H/H was maintained, 3) the chamber was returned to room air, and 4) room air was maintained. The animals were exposed to CIH/H for 8 h during the light phase and to normal air during the remaining 16 h. Air control animals were exposed to normal air (21% O2-79% N2) and placed adjacent to the chambers during the exposure period to undergo similar handling, general laboratory conditions, and background noise as the CIH/H group.

Slice preparation and electrophysiology. As previously described (10, 28, 29), we obtained brainstem slices containing the DMNX and ChR2-expressing PVN fibers for calcium imaging experiments and immunohistochemistry, as well as forebrain slices for electrophysiologic and immunohistochemistry (after 21 days of CIH/H exposure). Rats between P21 and P35 or P52-66, respectively, were anesthetized, killed, and transcardially perfused with ice-cold glycine-based artificial cerebrospinal fluid (aCSF) (252.0 mM glycine, 1.6 mM KCl, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 1.2 mM CaCl2, 18.0 mM NaHCO3, 11.0 mM glucose, 1.0 mM pyruvic acid, and 1.0 mM ascorbic acid, perfused with 95% O2-5% CO2, pH = 7.4) to achieve exsanguination. The brain was carefully removed, and brainstem slices (330 μm for confocal microscopy experiments, 200 μm for immunohistochemistry) were obtained using a vibratome (Leica VT1000S), whereas forebrain slices containing the PVN (400 μm for electrophysiology, 200 μm for immunohistochemistry) were obtained using a compressor (VF-300 from Precisionary Instruments). Slices were allowed to recover for 15 min at 32°C in an N-methyl-d-glucamine (NMDG)-based aCSF (110.0 mM NMDG, 2.5 mM KCl, 1.2 mM NaH2PO4, 25.0 mM NaHCO3, 25.0 mM glucose, 0.5 mM CaCl2, and 10.0 mM MgSO4; pH = 7.4, perfused with 95% O2-5% CO2), before being either transferred to 22–24°C aCSF (125 mM NaCl, 3 mM KCl, 2 mM CaCl2, 26 mM NaHCO3, 5 mM glucose, and 5 mM HEPES, perfused with 95% O2-5% CO2; pH = 7.4) in which electrophysiology experiments were performed, or to 10% formalin for immunohistochemistry.

Electrophysiology experiments were performed as previously described (10, 11, 28, 29) on a Zeiss Axioplan using a ¥40 water-immersion objective. Identified PVN neurons expressing mCherry were imaged with differential interference contrast optics, infrared illumination, and infrared-sensitive video detection cameras to gain better spatial resolution. Patch pipettes were filled with a solution at
pH 7.3 consisting of K-gluconic acid (150 mM), HEPES (10 mM), EGTA (10 mM), MgCl₂ (1 mM), and CaCl₂ (1 mM) to isolate for firing data to an electronic module. All rats with telemetry devices were allowed 7–14 days to recover from transmitter implantation surgery before any measurements were recorded. BP and HR were recorded via radio-frequency signals obtained through the Po-nemah data acquisition system (Data Sciences International). Mean arterial pressure (MAP) and HR in both groups of animals were recorded before CNO injection and 1 h after injection of CNO each day. Baseline recordings of BP and HR were obtained for 3 days before CIH/H exposure in both groups. Before and during the 28-day CIH/H exposure period, BP and HR were recorded daily for 20 min before CNO injection, 1 h after CNO injection, and for the entire 8-h duration of CIH/H exposure. Animals were killed with an overdose of the inhalation anesthetic isoflurane.

**Daily DREADDs activation.** After telemetry implantation (1–2 wk), DREADDs receptors on PVN OXT neurons were activated daily by intraperitoneal (IP) injection of CNO (1.0 mg/kg). To investigate the effects of CNO on resting BP and HR in both DREADDs-expressing and sham animals, 3 days before CIH/H exposure (control days), baseline BP and HR values were recorded for a 20-min period before CNO injection. Animals from each group then received an IP injection of CNO; BP and HR were recorded for 1 h, and the animals then underwent exposure to air for 8 h to achieve acclimation to the chambers. During the 21 days of CIH/H exposure, BP, and HR were recorded daily for 20 min before CNO injection, 1 h after CNO injection, with the last 15 min representing DREADDs activation, as DREADDs are activated 30–45 min after injection, and for the entire duration of CIH/H exposure. Both DREADDs and sham animals received the same regimen of daily injections of CNO.

**Statistical analysis.** Calcium responses from individual CHO cells were grouped into 0.5-s bins for a total of 10 bins with the fourth bin (time 0) representing the time of ChR2-expressing PVN fiber activation. Results are presented as a percentage of control, from bins 1, 2, and 3, before activation of ChR2-expressing PVN fibers, and statistically compared with data from the same experiment using a one-way ANOVA with repeated-measures followed by Bonferroni’s multiple-comparison test. For those experiments comparing CHO cell responses (time 0) in unexposed brainstem tissue to CIH/H exposure, a one-way ANOVA was used. Data with P < 0.05 was considered significant.

Changes in the action potential firing frequency were determined by the MiniAnalysis version 6.0.3 software (Synaptosoft) and grouped into 5-min bins for a total of 12 bins with the second bin representing the start of CNO application. Results are presented as mean ± SE and statistically compared with control data from the same experiment using a one-way ANOVA with repeated-measures followed by Bonferroni’s multiple-comparison test for all experiments. Data with P < 0.05 were considered significant.

To examine the effects of CNO on MAP, BP, and HR, as well as the chronic changes to MAP over the 21 days of CIH/H exposure, values were recorded for 20 min before and 1 h after CNO injection on three control days before CIH/H exposures and days 1, 3, 6, 9, 12, 15, 18, and 21 of CIH/H. On each of the three control days before CIH/H exposure, changes in MAP and HR before and after CNO injection were averaged in 15-min bins for analysis. Changes within the DREADDs-expressing animals and the sham animals were statistically analyzed by one-way ANOVA with repeated-measures followed by Bonferroni’s multiple-comparison test. To examine the statistical changes in MAP between the two groups of animals, a two-way ANOVA with repeated-measures followed by Bonferroni’s multiple-comparison test was used. Data with P < 0.05 were considered significant.

Software used for all statistical analysis of the data included GraphPad Prism 4.01 (GraphPad Software), MicroCal Origin 7.0 (OriginLabs), and Microsoft Excel (Microsoft).
RESULTS

Selectivity and in vitro activation of excitatory DREADDs in PVN OXT neurons. Selective excitatory DREADDs expression in PVN OXT neurons was achieved with two viral vectors, one expressing Cre under an OXT promoter (rAAV1-OXT-Cre) and the other a Cre-dependent vector expressing excitatory hM3D(Gq) DREADDs [AAV2-hSyn-DIO-hM3D(Gq)-mCherry]. As illustrated in Fig. 1, A–C, immunohistochemical analysis shows that this viral expression system elicited high (83.1 ± 2.1%) selectivity for DREADDs expression in OXT neurons. Viral DREADDs expression, as shown by the presence of mCherry, was limited to the PVN (Fig. 1, D and E).

The responses upon activation of DREADDs in PVN OXT neurons were assessed in vitro using the whole-cell patch-clamp method. The action potential firing frequency of DREADDs-expressing PVN neurons significantly increased within 5 min of CNO application (from 0.19 ± 0.05 Hz to 0.75 ± 0.14 Hz; n = 7; *P < 0.01; 1-way ANOVA; Fig. 2). As shown in Fig. 2, CNO application elicited long-lasting significant increases in the firing of DREADDs-expressing PVN OXT neurons.

The effects of CIH/H on OXT receptor activation. To examine whether the release of OXT from PVN fibers is altered with CIH/H and can be restored with chronic OXT neuron activation, the responses in OXT-sensitive CHO cells were examined in brainstem tissue from three groups of animals, air-exposed animals, CIH/H-exposed animals, and CIH/H-exposed animals accompanied with chronic activation of OXT neurons (treatment group). Photoactivation of ChR2-containing PVN fibers in the DMNX of brainstem slices from air-exposed animals evoked large, reproducible, and transient increases in intracellular calcium levels in OXT-sensitive CHO cells (increases to 1.21 ± 0.02 above baseline during the first second; n = 14; *P < 0.05; 1-way ANOVA with repeated measures; Fig. 3, bottom, left). These responses were similar to those shown previously using this approach, in which it was demonstrated that these responses can be evoked consistently and repetitively and are blocked by application of an OXT receptor antagonist.

Fig. 1. Subpopulation-specific adeno-associated viral (AAV) vectors express designer receptors exclusively activated by designer drugs (DREADDs) in hypothalamic paraventricular nucleus (PVN) oxytocin (OXT) neurons. Immunohistochemical staining of individual neurons in the PVN with OXT (A, green) and hSyn-DIO-hM3D(Gq)-mCherry expression (B, red) are shown. The merged image, C, is a typical example that illustrates selective (83.1 ± 2.1%) expression of DREADDs in PVN OXT neurons. Scale bar for A–C = 32 μm. As shown in D and E, viral DREADDs expression is limited to the PVN (scale bar in D and E = 250 μm).
ANOVA. 

Top-N-oxide (CNO) (29), configuration from a PVN OXT neuron expressing DREADDs before clozapine-N-oxide (29), configuration from a PVN OXT neuron expressing DREADDs before clozapine-N-oxide (29) recorded in current-clamp ADDs. Representative action potential traces (Fig. 2. In vitro activation of PVN OXT neurons expressing excitatory DREADDs activation with CNO application significantly increases the frequency of action potentials before and after CNO application in PVN OXT firing of PVN OXT neurons. Quantitative bar charts depict the average course effects of CNO administration during the hour imme-

Towards examining whether restoration of OXT neuron function during CIH/H could restore responses in OXT-sensitive CHO cells, PVN OXT neurons were activated daily before and during CIH/H by daily injections of CNO to activate PVN OXT neurons via excitation of DREADDs receptors in PVN OXT neurons (treatment group). In the treatment animals (CIH/H accompanied with chronic activation of OXT neurons), the responses in OXT-sensitive CHO cells upon photo-stimulation of ChR2-containing PVN fibers in the DMNX were restored and were not significantly different from responses in air-exposed animals (increases to 1.23 ± 0.03 above baseline during the first second; n = 17; *P < 0.05; 1-way ANOVA with repeated measures; Fig. 3, bottom, right). These restored responses in treatment animals were significantly increased compared with CIH/H-exposed animals (+P < 0.05; 1-way ANOVA; Fig. 3, bottom, right). These data indicate that OXT released from PVN fibers in the DMNX is diminished with CIH/H but that this release can be restored with chronic PVN OXT neuron activation.

Acute PVN OXT neuron activation decreases resting MAP and HR. To identify the in vivo functional consequences of chronic activation of OXT neurons with CIH/H, two additional groups of animals were instrumented with telemetry devices to record BP and HR in conscious unrestrained rats. The time course effects of CNO administration during the hour imme-

(29). In contrast to the robust responses in air-exposed animals, CHO cell responses upon PVN fiber stimulation in brainstem slices from animals exposed to CIH/H were significantly depressed (average increases to only 1.08 ± 0.01 above baseline during the first second; n = 16; *P < 0.05; 1-way ANOVA with repeated measures; Fig. 3, bottom, middle) compared with responses in air-exposed animals (+P < 0.05; 1-way ANOVA; Fig. 3, bottom, left). These results indicate that the release of OXT from PVN fibers in the DMNX is significantly decreased following CIH/H exposure.

To examine whether restoration of OXT neuron function during CIH/H could restore responses in OXT-sensitive CHO cells, PVN OXT neurons were activated daily before and during CIH/H by daily injections of CNO to activate PVN OXT neurons via excitation of DREADDs receptors in PVN OXT neurons (treatment group). In the treatment animals (CIH/H accompanied with chronic activation of OXT neurons), the responses in OXT-sensitive CHO cells upon photo-stimulation of ChR2-containing PVN fibers in the DMNX were restored and were not significantly different from responses in air-exposed animals (increases to 1.23 ± 0.03 above baseline during the first second; n = 17; *P < 0.05; 1-way ANOVA with repeated measures; Fig. 3, bottom, right). These restored responses in treatment animals were significantly increased compared with CIH/H-exposed animals (+P < 0.05; 1-way ANOVA; Fig. 3, bottom, right). These data indicate that OXT released from PVN fibers in the DMNX is diminished with CIH/H but that this release can be restored with chronic PVN OXT neuron activation.

Acute PVN OXT neuron activation decreases resting MAP and HR. To identify the in vivo functional consequences of chronic activation of OXT neurons with CIH/H, two additional groups of animals were instrumented with telemetry devices to record BP and HR in conscious unrestrained rats. The time course effects of CNO administration during the hour imme-
after CNO injection were analyzed. Both DREADDs-expressing and sham animals experienced significant increases in MAP and HR compared with baseline immediately following IP injection of CNO (changes in MAP in DREADDs-expressing animals: 104.0 ± 2.6 mmHg before CNO to 108.0 ± 2.4 mmHg immediately after CNO injection; n = 8, *P < 0.05, 1-way ANOVA, Fig. 4, top; in sham animals: 102.0 ± 3.3 mmHg before CNO to 115.0 ± 2.7 mmHg immediately after CNO injection; n = 7, +P < 0.0001, 1-way ANOVA, Fig. 4, top; changes in HR in DREADDs-expressing animals: 416.0 ± 7.1 beats/min before CNO to 439.0 ± 5.5 beats/min immediately after CNO injection, n = 8, *P < 0.05, 1-way ANOVA, Fig. 4, bottom; in sham animals: 394.0 ± 10.4 beats/min before CNO to 447.0 ± 11.1 beats/min immediately after CNO injection, n = 7, +P < 0.0001, 1-way ANOVA, Fig. 4, bottom). DREADDs-expressing animals, however, also experienced significant decreases in both MAP and HR compared with baseline 45 min after CNO injection, whereas there was no significant decrease in MAP or HR compared with baseline after CNO injection in sham animals (MAP 45 min after CNO injection, n = 8, *P < 0.0001, 1-way ANOVA, Fig. 4, top; in sham animals: 102.0 ± 3.3 mmHg before CNO to 100.0 ± 3.2 mmHg 45 min after CNO injection, n = 7, P > 0.05, 1-way ANOVA, Fig. 4, bottom). HR 45 min after CNO injection in DREADDs-expressing animals: 416.0 ± 7.1 beats/min after CNO injection to 362.0 ± 10.5 beats/min 45 min after CNO injection, n = 8, *P < 0.0001, 1-way ANOVA, Fig. 4, bottom; in sham animals: 394.0 ± 10.4 beats/min after CNO to 389.0 ± 9.8 beats/min 45 min after CNO injection, n = 7, P > 0.05, 1-way ANOVA, Fig. 4, bottom).

To test for any possible effects of CNO that were not DREADDs mediated, CNO was administered to non-DREADDs-expressing animals. CNO administration had no effect on MAP and HR in sham animals without DREADDs expression either before or after 21 days of CIH/H (MAP 45 min after CNO: sham animals: 416.0 ± 7.1 beats/min before CNO to 447.0 ± 11.1 beats/min immediately after CNO injection, n = 7, +P < 0.0001, 1-way ANOVA, Fig. 4, bottom). As anticipated, and shown in Fig. 5B, top, after 21 days of CIH/H, sham animals became hypertensive.

In contrast, however, CNO administration in DREADDs-expressing animals significantly decreased resting HR and MAP before and after 21 days of CIH/H exposure (MAP before CIH/H: 102.0 ± 3.3 mmHg before CNO to 100.0 ± 3.2 mmHg after CNO, n = 7, 1-way ANOVA, P > 0.05, Fig. 5B, top; MAP after CIH/H: 120.0 ± 0.5 mmHg before CNO to 122.0 ± 1.56 mmHg after CNO, n = 7, 1-way ANOVA, P > 0.05, Fig. 5B, top; HR prior to CIH/H: 394.0 ± 10.4 beats/min before CNO to 389.0 ± 9.8 beats/min after CNO, n = 7, 1-way ANOVA, P > 0.05, Fig. 5B, bottom). As anticipated, selective activation of PVN OXT neurons decreases resting BP and HR in conscious unrestrained telemetry-instrumented animals. These data indicate that selective activation of PVN OXT neurons decreases resting BP and HR in conscious unrestrained telemetry-instrumented animals. In addition, chronic activation of PVN OXT neurons prevented the increase in BP to hypertensive levels that occurred in sham animals.

Chronic PVN OXT neuron activation prevents the development of hypertension that occurs with CIH/H. To further characterize the extent and time course of the protective effects of OXT neuron activation with CIH/H, MAP and HR were examined throughout the 8-h period of CIH/H before and throughout 21 days of CIH/H exposure in sham and OXT neuron-activated animals. After 3 wk of CIH/H, MAP increased to hypertensive levels in sham animals (from a MAP of 102.0 ± 3.3 mmHg on control days to 120.0 ± 0.5 mmHg on day 21, n = 7, +P < 0.01, 1-way ANOVA with repeated measures, Fig. 6). Interestingly, animals receiving daily PVN OXT neuron activation experienced no significant changes in MAP throughout the 21 days of CIH/H exposure (from a MAP of 104.0 ± 2.6 mmHg on control days to 103.0 ± 3.0 mmHg on day 21, n = 8, P > 0.05, 1-way ANOVA with repeated measures, Fig. 6). Animals in the sham group experienced significant increases in MAP from day 12 through day 21,
CNO injections

beats/min on control days to 369.0 in DREADDs-expressing animals experienced a significant decrease in HR after 3 wk of CIH/H: MAP in sham group of 120.0 vs. 117.0 in DREADDs-expressing group of 103.0 mmHg; MAP in sham group of 120.0 vs. 117.0 in DREADDs-expressing group of 105.0 mmHg.

Fig. 5. Acute PVN OXT neuron activation decreases resting MAP and HR. The values for both MAP and HR in both groups of animals represent the averages of MAP and HR recorded 15 min before CNO injection and the period of 45–60 min after CNO injection, as DREADDs activation in vivo typically has a 30–45-min delay after intraperitoneal CNO injections. A: changes in resting MAP and HR before and after CNO injection in DREADDs-expressing animals before and after 21 days of CIH/H. MAP in DREADDs-expressing animals was significantly decreased 45 min after CNO injection (before CIH/H: n = 8, *P < 0.00015 vs. control, 1-way ANOVA, top left; after 21 days of CIH/H: n = 8, *P < 0.0054 vs. control, 1-way ANOVA, top right), and HR was also significantly decreased in DREADDs-expressing animals 45 min after CNO injection (before CIH/H: n = 8, *P < 0.0001 vs. control, 1-way ANOVA, bottom left; after 21 days of CIH/H: n = 8, *P < 0.0017 vs. control, 1-way ANOVA, bottom right). B: changes in resting MAP and HR before and after CNO injection in sham animals before and after 21 days of CIH/H. CNO injection before and after 21 days of CIH/H did not significantly change either MAP (before CIH/H: n = 7, P > 0.05, 1-way ANOVA, top left; after 21 days of CIH/H: n = 7, P > 0.05, 1-way ANOVA, top right) or HR (before CIH/H: n = 7, P > 0.05, 1-way ANOVA, bottom left; after 21 days of CIH/H: n = 7, P > 0.05, 1-way ANOVA, bottom right) in sham animals.

whereas the increase in MAP was prevented in the DREADDs-expressing animals (day 12: MAP in sham group of 117.0 ± 4.1 mmHg, MAP in DREADDs-expressing group of 105.0 ± 2.6 mmHg; day 15: MAP in sham group of 118.0 ± 2.6 mmHg, MAP in DREADDs-expressing group of 105.0 ± 3.1 mmHg; day 18: MAP in sham group of 122.0 ± 1.1 mmHg, MAP in DREADDs-expressing group of 104.0 ± 2.9 mmHg; day 21: MAP in sham group of 120.0 ± 0.5 mmHg, MAP in DREADDs-expressing group of 103.0 ± 3.0 mmHg; n = 7 sham animals and 8 DREADDs-expressing animals; *P < 0.01; 2-way ANOVA with repeated measures; Fig. 6). Sham animals experienced no significant changes in HR after 3 wk of CIH/H exposure (from 394.0 ± 10.3 beats/min on control days to 379.0 ± 15.4 beats/min on day 21, n = 7, P > 0.05, 1-way ANOVA with repeated measures, Fig. 6, bottom), whereas DREADDs-expressing animals experienced a significant decrease in HR despite CIH/H exposure (from 416.0 ± 7.1 beats/min on control days to 369.0 ± 11.1 beats/min on day 21, n = 8, +P < 0.01, 1-way ANOVA with repeated measures, Fig. 6, bottom). These data indicate that chronic activation of PVN OXT neurons prevents the development of hypertension that occurs in sham animals with CIH/H exposure.

Fig. 6. Chronic PVN OXT neuron activation diminishes the development of hypertension. Changes in MAP and HR from control days to day 21 of CIH/H exposure are shown. MAP in sham animals significantly increased from day 9 to day 21 compared with control days and reached hypertensive levels by day 12 (n = 7, +P < 0.0001 vs. control, 1-way ANOVA). MAP did not significantly increase in DREADDs-expressing OXT neuron-activated animals over the 21 days of CIH/H exposure compared with control days (n = 8, 1-way ANOVA). MAP significantly increased in sham animals compared with DREADDs-expressing animals from day 12 to day 21 (n = 7, +P < 0.0001, 2-way ANOVA with repeated measures). HR, bottom, was significantly decreased in DREADDs-expressing treatment animals on day 21 compared with control days (n = 8, +P < 0.0001, 1-way ANOVA), whereas HR in sham animals did not significantly change over the 21 days of CIH/H exposure (n = 7, 1-way ANOVA).

DISCUSSION

This study has four major findings: 1) the release of OXT from PVN fibers at parasympathetic brainstem targets is diminished following CIH/H exposure, 2) PVN OXT neuron activation during CIH/H exposure restores the release of OXT in the DMNX, 3) acute PVN OXT neuron activation decreases resting HR and MAP, and 4) chronic activation of PVN OXT neurons prevents the development of hypertension with CIH/H. Together, our data identify PVN OXT neuron activation as a potential novel and powerful target to mitigate the adverse cardiovascular consequences of OSA.

The results in this study identify an important pathway in which the release and function of OXT are altered with CIH/H...
OXYTOCIN NEURON ACTIVATION BLUNTS HYPERTENSION

exposure. The release of OXT upon optogenetic stimulation of PVN fibers in the DMNX activates OXT receptors on OXT-sensitive CHO cells (29), and this release is significantly blunted in animals exposed to 21 days of CIH/H. This release of OXT, however, is restored in those animals that received chronic activation of PVN OXT neurons during CIH/H. Previous work has shown that the release of OXT from PVN neurons mediates the paired pulse facilitation of excitatory neurotransmission to CVNs, and this paired pulse facilitation is absent in CIH/H-exposed animals (10, 29). These results together suggest that endogenous OXT release from PVN neurons in the DMNX plays important roles in exciting CVNs and in the long-term control of BP and HR. Although the release of OXT is blunted with CIH/H, if OXT neuron activity is chronically increased, the release of OXT is restored and the development of hypertension is prevented.

Our results with sniffer CHO cells indicate that there is reduced release of OXT in CIH/H animals, and this release is restored with chronic activation of PVN OXT neurons using excitatory DREADDs. Further work is needed to directly establish whether OXT peptide levels are diminished in PVN OXT neurons in CIH/H animals and/or synaptic corelease of OXT is blunted and how these alterations are restored by chronic excitation of PVN OXT neurons. There are a number of potential mechanisms for these changes that include activity-dependent and activity-independent changes in gene transcription, decreased translational efficiency (including microRNA-mediated mechanisms), and dysfunction in synaptic corelease mechanisms for OXT. Mechanisms possibly involved in OXT synthesis in the PVN include the transcription factor Egr-1, which is localization in OXT neurons of the PVN and is modulated by prolactin through a mitogen-activated protein kinase-dependent pathway (3). The microRNA miR-24 could also be involved, as it is an important regulator of OXT and has been shown to control both transcription and peptide levels of OXT (6). Previous work has shown that spontaneously hypertensive rats have decreased OXT mRNA expression in the hypothalamus (15). Dynamic modulation of the calcium-dependent actin cytoskeleton has also been shown to be involved in modulating OXT release (30), and this could be important for synaptic release independent of changes in peptide levels.

These data extend previous studies that have demonstrated that OXT excites CVNs in the DMNX, which would ultimately cause a reduction in HR (28, 29) and those studies that have shown that OXT modulates parasympathetic nervous system activity (12). The loss of OXT release in the DMNX with CIH/H, as demonstrated in these findings, would be expected to contribute to an overall reduction in parasympathetic activity to the heart. Chronic PVN OXT neuron activation likely reestablishes activation of parasympathetic activity and serves to prevent the adverse hypertension that occurs with CIH/H. Although our previous work has shown that one likely mechanism of these in vivo responses includes mediating paired pulse facilitation in the excitatory pathway from the PVN to parasympathetic CVNs, the potential presynaptic and postsynaptic sites and signaling pathways of action of OXT in this pathway that are altered with CIH/H remain unknown. Additionally, the in vivo activation of PVN OXT neurons may involve pathways to other neurons in the DMNX, as well as other PVN OXT pathways, such as the pathway to the nucleus tractus solitarius, a region essential for integrating cardiorespiratory sensory information (1, 16). It is also recognized that the role of OXT neuron activation in the chemoreceptor reflex is poorly understood, and one potential important location of activation of OXT neurons is on sites within the chemoreceptor reflex circuitry. Furthermore, in vivo activation of PVN OXT neurons could have increased circulating OXT levels, and this could also play a role in the in vivo responses.

Our data indicate that 21 days of chronic OXT neuron activation prevent the hypertension that occurs in sham animals exposed to CIH/H. This beneficial effect of OXT neuron activation is also supported by previous studies. OXT administration has been reported to have antihypertensive effects in spontaneously hypertensive male rats and, when administered postnatally, leads to BP decreases that persist into adulthood (27). Furthermore, daily peripheral administration of exogenous OXT in postnatal rats for 5 days resulted in decreases in BP lasting at least 2 mo (17). The mechanism by which OXT decreases BP in this prior study was not identified. It has been suggested, however, that the long-lasting reductions in BP may be mediated by the CNS because elevated OXT concentrations in the CSF were found in rats injected with OXT (26), and this mechanism is strongly supported by the results in this study.

In summary, the present results establish an antihypertensive role of OXT neurons in OSA-mediated cardiovascular disease. This work provides evidence that 21 days of CIH/H exposure diminish the release of OXT onto parasympathetic brainstem nuclei and that chronic activation of PVN OXT neurons during CIH/H restores OXT release onto these parasympathetic brainstem regions, decreases resting BP and HR, and blocks the development of hypertension that occurs in sham animals exposed to CIH/H. This work provides a foundation to test in clinical studies whether OXT administration or OXT neuron activation can be beneficial in treating patients with cardiovascular diseases such as OSA.

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AUTHOR CONTRIBUTIONS

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OXYTOCIN NEURON ACTIVATION BLUNTS HYPERTENSION


