Hypothalamic neurons as hypoxia sensors that facilitate the peripheral chemoreflex

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Abstract

An inappropriately elevated peripheral chemoreflex (PCR), originating from the carotid bodies, contributes to obstructive sleep apnea (OSA) and associated cardiovascular diseases such as hypertension and heart failure. Hypothalamic orexin and corticotropin-releasing hormone (CRH) neurons participate in the control of breathing, including the PCR-mediated hypoxic ventilatory response (HVR). However, it is unclear whether these neurons can be intrinsically activated by hypoxia or if their activation is downstream of carotid body excitation. We hypothesize that orexin and CRH neurons are activated by hypoxia and contribute to the HVR in the absence of carotid body input. Using adult rats, we will perform a carotid sinus nerve (CSN) section or sham surgery. Rats will then be exposed to hypoxia (10% inspired O_2) for 2 hrs to allow expression of Fos (indicating activation) while recording respiration. Tissues will then be fixed and sectioned, followed by immunohistochemistry to quantify Fos immunoreactivity (IR). We expect that, compared to rats exposed to room air, hypoxia will increase the number of orexin and CRH neurons displaying Fos-IR, even in the absence of inputs from the CSN. We further expect that rats with sectioned CSNs will retain a significant HVR. These results would indicate that orexin and CRH neurons can be intrinsically activated by hypoxia and contribute to the PCR independently of carotid body input. This research will provide new mechanistic insight into the role of the hypothalamus in the PCR, information that could be harnessed to develop therapies treating OSA, hypertension and associated cardiovascular disease.

Introduction

Several cardiovascular diseases are associated with increased sensitivity of the peripheral chemoreflex (PCR) that originates from the carotid bodies. It is known that orexin contributes to the PCR-mediated hypoxic ventilatory response (HVR) and that orexin neurons are activated (Fos-immunoreactive) by hypoxia. Orexin neurons project to the paraventricular nucleus of the hypothalamus (PVN) and nucleus of the solitary tract (nTS), two nuclei integral to the HVR **(Fig. 1)**. Orexin and PVN neurons with projections to the nTS are activated by acute hypoxia. The majority of hypoxia-activated PVN neurons are also immunoreactive (IR) for corticotropin-releasing hormone (CRH). It is unknown whether the activation of orexin and PVN neurons is the result of being part of the PCR neural circuitry or, alternatively, because they are intrinsically-activated by hypoxia. **Our proposed experiments address the hypothesis that these neurons are activated by hypoxia and contribute to the HVR even in the absence of input from the carotid bodies.**

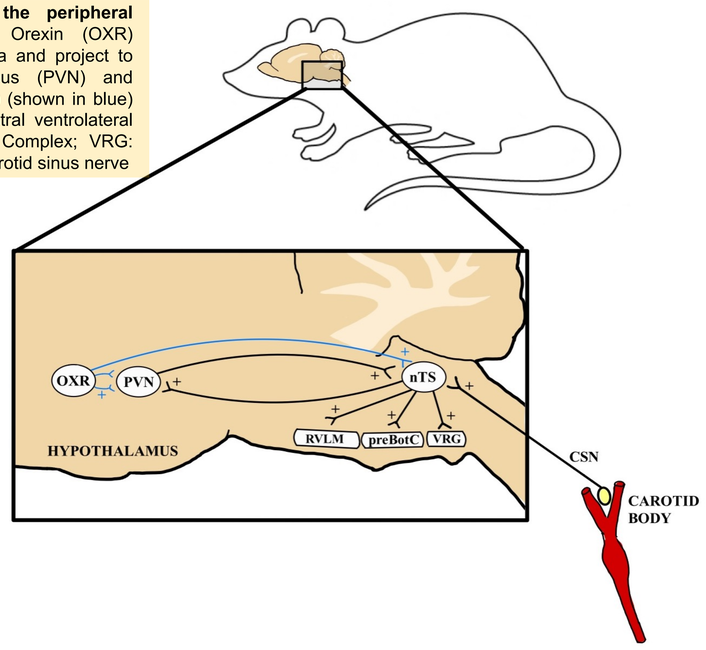

Fig. 1. Diagram illustrating the peripheral chemoreflex (PCR) in rats. Orexin (OXR) neurons are activated by hypoxia and project to both the paraventricular nucleus (PVN) and nucleus of the solitary tract (nTS) (shown in blue) to facilitate the PCR. RVLM: rostral ventrolateral medulla; preBotC: preBotzinger Complex; VRG: ventral respiratory group; CSN: carotid sinus nerve

Hypothesis

Orexin and corticotropin-releasing hormone neurons are activated by hypoxia and contribute to the hypoxic ventilatory response even in absence of carotid sinus nerve input.

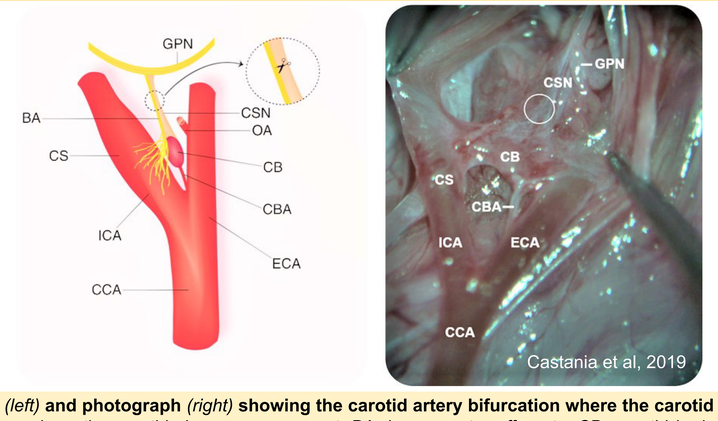

Fig. 2. Drawing *(left)* and photograph *(right)* showing the carotid artery bifurcation where the carotid bodies reside. The white circle shows where the carotid sinus nerve was cut. BA, baroreceptor afferents; CB, carotid body; CBA, carotid body artery; CCA, common carotid artery; CS, carotid sinus; CSN, carotid sinus nerve; ECA, external carotid artery; GPN, glossopharyngeal nerve; ICA, internal carotid artery; and OA, occipital artery

Methods

Animals

Male Sprague Dawley rats (age 2 months) are deeply anesthetized with isoflurane (4.5% induction, 3-3.5% maintenance). A ventral midline incision is made along the neck from the chin to the sternum. The muscle and fascia are bluntly dissected to visualize the carotid bifurcation and expose the carotid sinus nerve (CSN), which are cut bilaterally (CSNx). Sham rats have identical surgical procedures without CSNx. Rats are allowed to recover for 2-3 days before testing the peripheral chemoreflex while conscious.

Chemoreflex testing

All experiments were done during the inactive (light) phase. Whole body plethysmography is used to measure the ventilation of sham and CSN rats during baseline (normoxic) conditions and in response to hypoxia, with inspired O_2 taken from 21% to 11% over 3-5 min. In some rats, hypoxia will be maintained for 2 hours before returning to normoxic conditions to allow for expression of Fos.

Immunohistochemistry

Brains from CSNx and shame rats exposed to 2 hr hypoxia will be fixed in 4% PFA. Sections will be made from the brainstem and hypothalamus. Routinely used immunohistochemistry protocols will identify Fos in corticotropin-releasing hormone and orexin neurons. Fos+ neurons will be counted and any statistically significant differences between the two groups will be determined.

Results

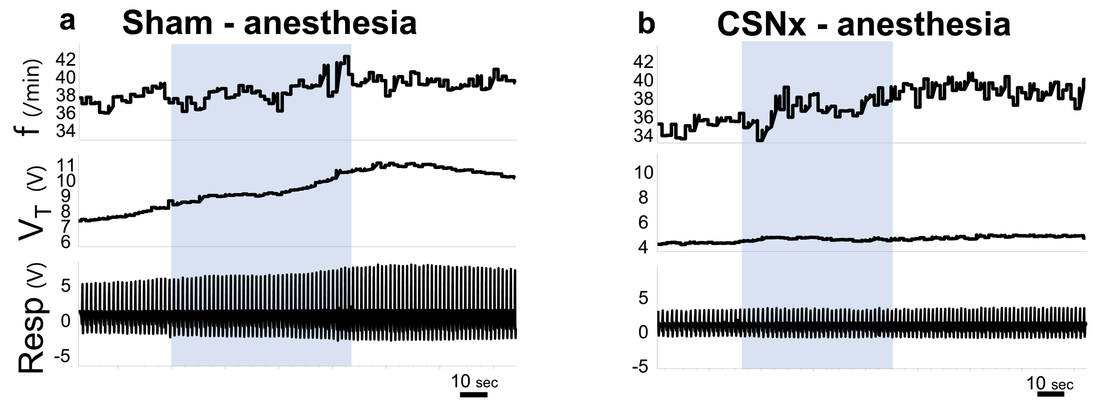

Fig. 3. Hypoxic ventilatory responses of a sham *(a)* and carotid sinus nerve sectioned (CSNx) rat *(b)* to hypoxia while under isoflurane anesthesia. Note that the CSNx rat has a smaller tidal volume (V_T) response but a similar frequency response (f) compared to the CSNx rat. Resp= raw respiratory trace. Period of hypoxia indicated by blue shading.

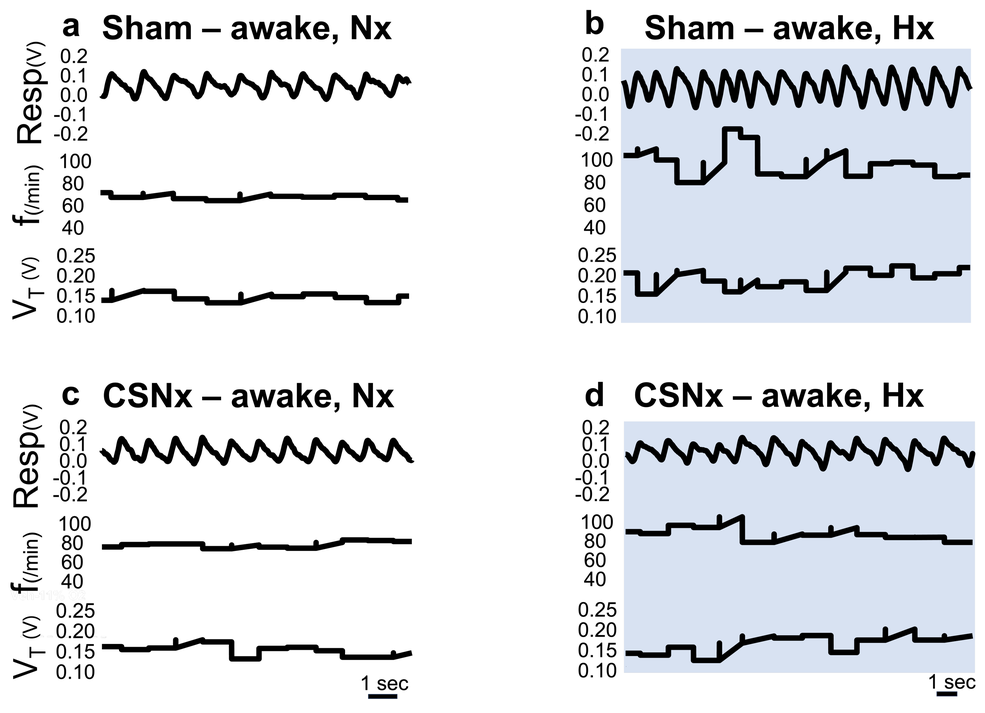

Fig. 4. Hypoxic ventilatory response of conscious sham and carotid sinus nerve sectioned (CSNx) rat to hypoxia. Shown is respiration in normoxia (Nx; *a* and *c*) and during hypoxia (Hx; *b* and *d*). Same variables shown as in Fig. 3. Note that CSNx rat displays a small increase in frequency during Hx, despite the CSN sectioning.

Conclusions

1. Carotid sinus nerve (CSN) denervation effectively reduces the hypoxic ventilatory response (HVR), mostly the tidal volume response
2. Whether conscious or anesthetized, CSN denervation does not completely eliminate the HVR, with a small frequency response persisting
3. Future immunohistochemistry experiments will assess whether orexin and corticotropin-releasing hormone neurons are still activated by hypoxia, and if the HVR that remains in conscious CSN sectioned rats is eliminated by Ox1R blockade.

References and Acknowledgements

Castania, JA, Katayama, PL, Brognara, F, Moraes, DJA, Sabino, JPJ, Salgado, HC. Selective denervation of the aortic and carotid baroreceptors in rats. Experimental Physiology. 2019; 104: 1335– 1342.

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