The Journal of Physiology

CAROTID BODY HYPERSENSITIVITY IN INTERMITTENT HYPOXIA AND OBSTRUCTIVE SLEEP APNEA

Nanduri R. Prabhakar, Ying-Jie Peng, and Jayasri Nanduri
DOI: 10.1113/JP284111

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Rodrigo Iturriaga (Referee #1)

Review Timeline:

Submission Date:	26-Jan-2023
Editorial Decision:	15-Feb-2023
Revision Received:	17-Mar-2023
Accepted:	29-Mar-2023

Senior Editor: Laura Bennet

Reviewing Editor: Silvia Conde

Transaction Report:

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Dear Dr Prabhakar,

Re: JP-SR-2023-284111 "CAROTID BODY AND SLEEP APNEA" by Nanduri R. Prabhakar, Ying-Jie Peng, and Jayasri Nanduri

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert referees and we are pleased to tell you that it is acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

The referee reports are copied at the end of this email.

Please address all the points raised and incorporate all requested revisions or explain in your Response to Referees why a change has not been made. We hope you will find the comments helpful and that you will be able to return your revised manuscript within 4 weeks. If you require longer than this, please contact journal staff: jp@physoc.org.

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We look forward to receiving your revised submission.

If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

The manuscript by Prof. Nanduri Prabakar is a very well written, concise and focused review on the cellular, molecular and epigenetic mechanisms contributing to the increased carotid body chemosensitivity in intermittent hypoxia and its relationship with obstructive sleep apnea and associated hypertension. The manuscript was reviewed by 2 experts in the field, and both highlighted the importance and the impact to the field.

Both reviewers made some minor suggestions that I believe that could be easily addressed, particularly in terms of manuscript organization. Note also, that the second reviewer suggests an alteration to manuscript title to better reflect the manuscript content.

REFEREE COMMENTS

Referee #1:

Prabhakar et al. reviewed the contribution of the carotid body (CB) in pathological consequences produced by obstructive sleep apnoea (OSA). This review focused how OSA causes CB-dependent increase in sympathetic nerve activity and hypertension in rodent models exposed to intermittent hypoxia, which is a hallmark of OSA, They discussed the underlying cellular, molecular, and epigenetic mechanisms. In addition, authors discussed emerging evidence for how CB afferents trigger cellular and molecular changes in the central and efferent chemoreflex pathway elicited by intermittent hypoxia.

The work of Prabhakar and colleagues have revealed the major impact of the CB to the pathological mechanisms in OSA. Their work was pioneer in the field. The topic is especially physiological important and highly clinically relevant. Overall, the review it is well-written and easy to followed. The discussion of the results obtained by Prabhakar´s and others groups is balanced and provide insight into physiological mechanisms in this field. This will be an important review article in the field. However, there are some points that authors should consider improving the Ms.

The organization of the article could be very much improved. The review started with a general introduction, followed by a discussion of the contribution of the CB chemoreflex OSA in a murine model (See Enhanced carotid body chemoreflex as a cause of OSA) with a deletion genetic deletion of the heme oxygenase-2 (HO-2), which catalyses carbon monoxide production (HO-2 null mice). However, from a historical point of view, several studies have shown that OSA patients and animals exposed to IH showed enhanced ventilatory and cardiovascular responses induced by hypoxia attributed to a potentiation of the CB chemosensory responses to hypoxia. Moreover, it is well known that IH enhances the hypoxic ventilatory response in rodents and cats, producing long-term facilitation of respiratory motor activity. The idea that CB chemoreceptors participated in the progression of the hypertension induced by CIH was initially proposed by Fletcher and colleagues. They found that bilateral CB denervation prevents the development of hypertension in rats exposed to CIH for 35 days, indicating that CB chemoreceptors were essential for the progression of the hypertension induced by CIH.

Moreover, recording of the CB chemosensory discharge have showed that exposure of animals to IH increases the basal CB discharge and enhances the chemosensory response to acute hypoxia. Indeed the pioneer work of Peng et al. (Proc. Natl. Acad. Sci. USA. 2003) found that basal CB discharges and chemosensory responses to acute hypoxia were higher in rats exposed to a pattern of CIH of short cyclic hypoxic episodes followed by normoxia. Similarly, Rey et al (J Physiol 2004) reported that cats exposed to CIH show enhanced CB chemosensory and ventilatory responses to acute hypoxia. Moreover, in vitro recording of the chemosensory discharge from isolated CBs showed that the potentiation of the hypoxic response is not due to systemic changes induced by IH.

The topics of "IH activates the carotid body" and "Carotid body as a mediator of OSA-associated hypertension", in this reviewer's opinion, should be stated up front of the discussion, and possible mechanisms for CB overactivation (HO-2 and CO) must be discussed later in the section "Mechanism(s) underlying carotid body activation by IH".

In the section of "How might ROS mediate carotid body activation by IH? Authors may discuss the possible effects of ROS on the mitochondrial respiratory chain, recently proposed by the Lopez-Barneo group.

Figures are adequate.

***

Referee #2:

GENERAL COMMENTS

This is an important review about the great contribution of Professor Nanduri Prabhakar´s laboratory for the understanding of cellular, molecular and epigenetic mechanisms underlying the increased sensitivity observed in the cells of the carotid body in experimental models of intermittent hypoxia.

In the first part of the review the authors addressed the possible role of hypersensitivity of carotid body to hypoxia as a cause of obstructive sleep apnea (OSA). For this purpose, they explored a large body of experimental evidences from their own laboratory obtained in the murine model of OSA (HO-2 null mice), which seems to be the best experimental models available for translational studies related to OSA. The combination of HO-2 and CSE null mice, in which the enhanced carotid body sensitivity is blunted make this model a raising star in this very important field. In this review the authors explored in details these experimental models and the strong arguments presented in favor of these models as the most appropriated for studies related to OSA are based upon their previous experimental data.

In the second part, the authors discusses how OSA causes carotid-body increase in sympathetic nerve activity (SNA) and hypertension with focus on the experimental model of intermitent hypoxia (IH) in mice. This part of the review is also very strong with respect to the mechanisms underlying the increased sensistivity of the cells in the carotid body, which may impact on the hightened SNA and hypertension. In order to avoid possible misinterpretation that the generation of sympathetic overactivity is restricted to changes in the carotid body cells of mice submitted to IH and also to enrich this important part of the review, I am suggesting the authors to add a new paragraph with additional information about possible changes that may also occur at the level of the neural pathways in the brainstem involved with the autonomic and respiratory control that might also impact in the overall sympathetic overactivity and hypertension observed in these experimental models of IH.

This is an exciting review about the data obtained in the recent past mostly in the laboratory of Professor Naduri Prabhakar and I suggest the authors to add, at the end of the review, a short chapter about the perspectives for new methods and approaches in IH and OSA investigation in the next few years. I am sure that the bright comments by the authors will enlight the pathways of the readers involved with this field.

SPECIFIC COMMENTS

The current title is too generic and it is not fully representative of the content of the very important review, which is mainly related to the experimental model of OSA and IH. For this reason, I suggest the authors to consider the following possibility for the title: "Carotid body hypersensitivity in experimental model of intermittent hypoxia". In fact, the review is correctly addressing the model of IH rather than the OSA in patients, which causes and mechanisms seems to be different of those observed in the murine experimental model.

The idea of "amalgamate" the literature on humans with experimental models of IH is interesting but it requires a clear distinction that in spite the fact that IH is a common feature in both pathophysiological conditions, there are several differences among OSA in humans and IH in mice that make a direct translational approach quite difficult for the readers. For this reason, I am also suggesting the authors to add a paragraph to discuss the similarities and mainly the differences of the OSA in humans and the murine model of IH in order to make sure for the readers this very important issue. This new paragraph may also add additional comments to make clear that the murine model of HO-2 null mice seems to be the most appropriated model for translational studies related to the OSA.

The sentence starting at the 4th line below the subtitle "Intermittent hypoxia (IH) is major stimulus for SNA activation and hypertension" (page 6): "First, rodents subjected to IH patterned after blood O2 dessaturations in OSA subjects alone was suficiente for producing hypertension and elevated SNA", is too important in the context of this review but it is not clear enough to express the complex ideas presented by the authors. For this reason, these concepts should be better discussed by the authors.

The Figure 2 is a simple schematic representation that is not helping much the readers to understand where in the brainstem the increase in the SNA is processed or altered. Considering a large number of studies in the literature related to the central pathways of the chemoreflex, the authors should explore these pathways and discuss how these changes are generated and highlight this complex neural network as another possible sites for the observed changes in generation and modulation of SNA.

_____

END OF COMMENTS

Confidential Review **26-Jan-2023**

PONT-BY POINT RESPONSES TO EDITOR AND REVIEWERS COMMENTS

We thank the Editor and reviewers for the constructive comments. Below are the point by point-by-point responses. All revisions are highlighted in RED.

EDITORIAL COMMENT

Comment: *Both reviewers made some minor suggestions that I believe that could be easily addressed, particularly in terms of manuscript organization. Note also, that the second reviewer suggests an alteration to manuscript title to better reflect the manuscript content.*

Response: We reorganized the MS as suggested by reviewers and changed the title as suggested by Reviewer 2

REFEREE#1

Comment: *The topics of "IH activates the carotid body" and "Carotid body as a mediator of OSA-associated hypertension", in this reviewer's opinion, should be stated up front of the discussion, and possible mechanisms for CB overactivation (HO-2 and CO) must be discussed later in the section "Mechanism(s) underlying carotid body activation by IH".*

Response: We re-arranged the MS as suggested.

Comment: *In the section of "How might ROS mediate carotid body activation by IH? Authors may discuss the possible effects of ROS on the mitochondrial respiratory chain, recently proposed by the Lopez-Barneo group.*

Response: We included Lopez-Barneo's study with inducible deletion of mitochondrial complex I (**Page 7; para 2; Lines 9-22**).

REFEREE#2:

Comment: *I am suggesting the authors to add a new paragraph with additional information about possible changes that may also occur at the level of the neural pathways in the brainstem involved with the autonomic and respiratory control that might also impact in the overall sympathetic overactivity and hypertension observed in these experimental models of IH.*

Response: We added two paragraphs briefly describing neural pathways associated with CB chemoreflex dependent excitation of sympathetic nervous system by IH **(Pages 11-12).**

Comment: *I suggest the authors to add, at the end of the review, a short chapter about the perspectives for new methods and approaches in IH and OSA investigation in the next few years.*

Response: We added additional section "**Gaps in the knowledge and future directions**" (Page 16 short paragraph outlining potential future studies **(last paragraph on Pages 16-17).**

Comment: *The current title is too generic and it is not fully representative of the content of the very important review, which is mainly related to the experimental model of OSA and IH. For this reason, I suggest the authors to consider the following possibility for the title: "Carotid body hypersensitivity in experimental model of intermittent hypoxia".*

Response: As suggested we changed the title as “CAROTID BODY HYPERSENSITIVITY IN INTERMITTENT HYPOXIA AND OBSTRUCTIVE SLEEP APNEA”

Comment: *I am also suggesting the authors to add a paragraph to discuss the similarities and mainly the differences of the OSA in humans and the murine model of IH in order to make sure for the readers this very important issue. This new paragraph may also add additional comments to make clear that the murine model of HO-2 null mice seems to be the most appropriated model for translational studies related to the OSA.*

Response: We added this information as suggested on **Page 17; Para 1; Lines 5-7.**

Comment: *The sentence starting at the 4th line below the subtitle "Intermittent hypoxia (IH) is major stimulus for SNA activation and hypertension" (page 6): "First, rodents subjected to IH patterned after blood O2 dessaturations in OSA subjects alone was suficiente for producing hypertension and elevated SNA", is too important in the context of this review but it is not clear enough to express the complex ideas presented by the authors. For this reason, these concepts should be better discussed by the authors.*

Response: We have re-written this section **(Page 6; Para 1; line5-9).**

Comment: *The Figure 2 is a simple schematic representation that is not helping much the readers to understand where in the brainstem the increase in the SNA is processed or altered. Considering a large number of studies in the literature related to the central pathways of the chemoreflex, the authors should explore these pathways and discuss how these changes are generated and highlight this complex neural network as another possible sites for the observed changes in generation and modulation of SNA.*

Response: Modified Figure 2 as suggested.

Dear Dr Prabhakar,

Re: JP-SR-2023-284111R1 "CAROTID BODY HYPERSENSITIVITY IN INTERMITTENT HYPOXIA AND OBTRUCTIVE SLEEP APNEA" by Nanduri R. Prabhakar, Ying-Jie Peng and Jayasri Nanduri

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Yours sincerely,

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EDITOR COMMENTS:

Reviewing Editor:

The manuscript by Dr. Prabhakar is a well structured and clear revision on the relation between carotid body hypersensitivity and obstructive sleep apnea/intermittent hypoxia, providing also new future directions to the field. It will be highly influential.

REFEREE COMMENTS:

Referee #1:

This revised Ms is suitable for publication.

Referee #2:

The revised version of this manuscript by Prabhakar et al. presents several changes, which are in accordance with the comments and suggestions by the referees. The authors altered the order of the topics and correspondent figures in the

revised version and the new text is now flowing much better than the original. In my view the most important adds to the text were: a) the new Introduction, making clear the goals of the review, and b) the new topics "Effect of IH on central component of the carotid body chemoreflex", "Stimulus for OSA associated SNA activation and hypertension" and "Gaps in the knowledge and future directions". In the last new topic, it is relevant the hypothesis presented by the authors that hyperactive chemoreflex by stimulating breathing lower arterial CO2, which may reduce the excitability of hypoglosssal motoneurons leading to airway collapse and causing obstructive sleep apnea. Some new and important citations were added to the revised version contributing to clarify several issues identified in the original version of the manuscript.

1st Confidential Review **17-Mar-2023**