ISAC XXI Virtual Kickoff Meeting

International Society of Arterial Chemoreception

(Mal) adaptive responses of peripheral chemoreceptors: O_2 dependent and independent mechanisms

28th June, 1pm GMT

13.00 – 13:15 Opening session – Emilia Monteiro (ISAC President, NOVA Medical School, Portugal)

13:15 – 14:00 "Sleep apnea and intermittent hypoxia: unmet needs and challenges for biomedical research" Jean Louis Pepin (Service EFCR/Pneumologie, CHU de Grenoble site Nord - Hôpital Albert Michallon, Grenoble, France)

14:00 – 16:00 Presentations from Young Scientists (Undergraduate, Master, PhD and Young Post-Docs)

***Moderator:** Silvia V. Conde*

***14.00** Steady-State chemoreflex drive captures ventilatory acclimatization during ascent to altitude effect of Diamox*

Authors: Cates V, Bruce CD, Saran G, Leacy JK, O'Halloran KD, Brutsaert TD, Sherpa MT, Day TA

***14.15** The cardiorespiratory disturbances induced by social isolation in adult male rats: a comparison with intermittent hypoxia*

Authors: Marianne Gagnon, Stephanie Fournier, Francois Marcouiller, Danuzia Ambrozio-Marques, Vincent Joseph and Richard Kinkead

14.30 *A bet in olive oil: hydroxytyrosol reverts increased blood pressure and cysteine disulfides induced by chronic intermittent hypoxia.*

Authors: M. João Correia, Elsa Mecha, Catarina O. Sequeira, Nuno R. Coelho, Teresa Serra, Sandra Silva, Judit Morello, Maria R. Bronze, Emília C. Monteiro, Sofia A. Pereira.

14.45 *Sympathetic Control of Carotid Body Excitability in Spontaneously Hypertensive Rats*

Authors: Igor S. A. Felippe, Tymoteusz Zera, Melina P. da Silva, Davi J. A. Moraes, Fiona McBryde, Julian F. R. Paton

15.00 *Role of Leptin-TRPM7 Signaling in Carotid bodies in the Pathogenesis of Sleep-Diordered Breathing in Obesity.*

Authors: Lenise J. Kim, Mi-Kyung Shin, Huy Pho, Nishitha Hosamane, Frederick Anokye-Danso, Rexford S. Ahima, Luu Pham, and Vsevolod Y. Polotsky

15.15 *Oxygen Regulation of Breathing is Restored by NDI1 Expression in MCI-Deficient Mice.*

Authors: Blanca Jiménez-Gómez, Patricia Ortega-Sáenz, Lin Gao and José López-Barneo

15.30 *Preliminary Observations of Chemosensitive Thermal Microdomains in Rat Carotid Body Type I Cells*

Authors: R. J. Rakoczy, C. Schiebrel, & C. N. Wyatt

15.45 *Beyond the output: the spinal cord's role in hypoxia sensing - Spinal Oxygen Sensors*

Authors: Nicole O. Barioni, Fatemeh Derakhshan, Luana Tenorio Lopes, Hiroshi Onimaru, Arijit Roy, Fiona McDonald, Erika Scheibli, Mufaddal I. Baghdadwala, Negar Heidari, Manisha Bharadia, Keiko Ikeda, Itaru Yazawa, Yasumasa Okada, Michael B. Harris, Mathias Dutschmann, Richard J.A. Wilson

16:00 *Final Remarks and Farewell*

Awards will be announced in the webpage and communicated directly to the presenters.

Scientific Committee
Emília C. Monteiro
Sílvia V. Conde
Estelle Gauda

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STEADY-STATE CHEMOREFLEX DRIVE CAPTURES VENTILATORY ACCLIMATIZATION DURING ASCENT TO ALTITUDE: EFFECT OF DIAMOX

International Society of Arterial Chemoreception
Virtual Kick off Meeting
June 28, 2021

Authors:
Cates V, Bruce CD, Saran G, Leacy JK, O'Halloran KD, Brutsaert TD, Sherpa MT, Day TA

ABSTRACT

Ventilatory acclimatization of respiratory chemoreflexes is important during high altitude ascent to protect oxygenation. Transient respiratory gas tests fail to capture the integrated steady-state responses to chronic hypoxic exposure in high altitude fieldwork. We recently characterized a novel index of steady-state respiratory chemoreflex drive (SS-CD), accounting for integrated contributions from central and peripheral respiratory chemoreceptors during steady-state breathing. Diamox is utilized during ascent for prevention or treatment of acute mountain sickness (AMS), eliciting metabolic acidosis, stimulating respiratory chemoreceptors, and further protecting oxygenation. To determine if SS-CD reflects both ventilatory acclimatization and perturbations in acid-base balance during ascent to high altitude, we characterized SS-CD in two groups of native lowlanders during ascent to 4240m over seven days - one group taking Diamox (D; 125 mg BID; n=12) and one group with no Diamox (ND; n=22). At 1130/1400m (day zero) and 4240m (day seven), steady-state measurements of resting ventilation ($\dot{V}_I$; L/min), pressure of end-tidal (P_{ET})CO_2 (Torr), peripheral oxygen saturation (SpO_2; %) and a stimulus index (SI; $P_{ET}CO_2/SpO_2$) were calculated. SS-CD was then calculated by indexing $\dot{V}_I$ against SI. With ascent to 4240m in both conditions, $\dot{V}_I$ increased, $P_{ET}CO_2$ and SpO_2 were lower, and SS-CD increased, indicating ventilatory acclimatization ($P<0.05$). In the D group, $P_{ET}CO_2$ was lower and SpO_2 and SS-SD were all higher at 4240m ($P<0.05$) compared to the ND group, suggesting that Diamox-induced metabolic acidosis augmented ventilatory acclimatization. SS-CD may have utility in assessing ventilatory acclimatization during prolonged stays at altitude, providing an alternative to complex and confounded transient chemoreflex tests.

Funding Sources: Natural Sciences and Engineering Research Council of Canada, Alberta Innovates Health Solutions and Alberta Government Student Temporary Employment Program.

TITLE: The cardiorespiratory disturbances induced by social isolation in adult male rats: a comparison with intermittent hypoxia

AUTHORS: Marianne Gagnon, Stéphanie Fournier, François Marcouiller, Danuzia Ambrozio-Marques, Vincent Joseph and Richard Kinkead

AFFILIATION: Centre de Recherche de l'Institut Universitaire de Cardiologie et Pneumologie de Québec. Faculté de Médecine. Université Laval. Québec, QC. Canada

ABSTRACT:

Introduction: Social isolation (SI) is an established risk for disease and the COVID-19 pandemic has brought the entire world to appreciate its impact on mental and physical health. Although the impact of social deprivation on mental health is well established, we have little information on its potential effect on cardiorespiratory homeostasis. Hypertension and obesity increase vulnerability to COVID-19, but they are also important co-morbidities of sleep apnea. In social mammals, deprivation of social contact causes significant psychological stress and is sufficient to induce hypertension. Here, we revisited this observation and tested the hypothesis that in male rats, social isolation induces cardiorespiratory and metabolic disturbances similar to those reported in sleep apnea patients. We then compared our results to animals exposed to intermittent hypoxia (IH), the main animal model of sleep apnea.

Methodology: Adult (8 weeks old) male rats were subjected to either three weeks of social isolation (SI) or standard care. Social isolation (SI) consisted of single housing without visual contact with other rats (n = 13); the control rats were housed in pairs (n = 12), both groups under normoxic conditions. The third group (n = 12) housed in pairs was exposed to intermittent hypoxia (IH: FiO_2 = 0.10 – 30s, 10 cycles/hour, 8 hours/day, 7 days). Cardiovascular measurements were obtained by the tail cuff pressure method, and quantification of apneas was performed with whole body plethysmography. The body composition analysis was assessed by preclinical magnetic resonance imaging. Results were then analyzed with ANOVA to assess the effects of time and treatment on cardiorespiratory and metabolic outcomes.

Results: Over the course of the 21-day protocol, rats subjected to SI gained marginally more weight that controls (6,2%). However, their body composition contained 19,7% more fat and 11,3% more fluids. Yet food consumption did not differ between groups. At the end of the protocol, SI rats were hypertensive (mean arterial pressure 15 mmHg higher than controls). The apnea index during sleep of SI rats (19 ± 1.6 events/h) was higher than controls (13 ± 3.7 events/h). Both SI and IH animals showed longer apneas than controls. After exposition, rodents subjected to IH lost weight and drastically reduced their food consumption, resulting in 23,7% less weight gain than controls and lower food intake (CRTL: 0,312 ± 0,005 g/day/100g, IH: 0,123 ± 0,020g/day/100g). Unlike SI rats, animals exposed to IH had a 30,4% decrease in body fluid weight compared to controls. The mean arterial pressure of IH rats was 7 mmHg higher than controls and they exhibited tachycardia with a heart rate greater than 54 bpm compared to controls.

Discussion: The increase in apnea frequency during sleep is an indication of an abnormality in respiration regulation as seen in sleep apnea patients. Since hypertension and obesity are important risk factors for complications in case of COVID-19 infection, our results suggest that social isolation is an aggravating factor in males. Our results demonstrate that an apparently modest stress is sufficient to induce cardiorespiratory disorders similar to those observed following IH. However, the metabolic disturbance resulting from social isolation is more in line with the phenotype observe in patients with sleep apnea. Since preliminary observation indicate that the effects of SI are not observed in females, we propose that SI is a valuable model to investigate specific aspects of pathophysiology of sleep apnea related to social distress and their sex-based differences.

A bet in olive oil: hydroxytyrosol reverts increased blood pressure and cysteine disulfides induced by chronic intermittent hypoxia

M. João Correia[1], Elsa Mecha[2], Catarina O. Sequeira[1], Nuno R. Coelho[1], Teresa Serra[2], Sandra Silva[2], Judit Morello[1], Maria R. Bronze[2,3], Emília C. Monteiro[1], Sofia A. Pereira[1]

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It is widely accepted that the desaturation/reoxygenation cycles present in chronic intermittent hypoxia (CIH) are a major contributor to arterial hypertension (HTN) associated with obstructive sleep apnea (OSA). This particular type of HTN is more difficult to control [1,2], demanding for novel preventive strategies. For instance, the consumption of olive oil has demonstrated blood pressure (BP)-lowering effects [3,4], mainly associated with antioxidant/anti-inflammatory mechanisms. However, this is still controversial, as several clinical trials have failed to prove that antioxidants *per se* are effective in preventing/treating HTN. We have been investigating the impact of CIH in oxidative dynamics of cysteine (Cys) in kidney [5], since cysteine disulfides (free oxidized cysteine metabolites, as cystine) are emerging as non-free radical species driving to oxidative stress with clinical importance. We hypothesized that the polyphenol of extra-virgin olive oil – hydroxytyrosol (OHTyr) - may provide a useful alternative to free radical scavengers as means to prevent and manage OSA-HTN.

Herein, we used an animal model of mild OSA (5.6 CIH cycles/h, 10.5 h/day during their inactive period) in which rats become hypertensive following 21 days of exposure [1]. Systolic and diastolic BP (SBP and DBP) were measured twice a day (8am and 6pm) using telemeters placed surgically in the abdominal aorta. A first set of animals was used to investigate if the increase in BP induced by CIH is prevented by OHTyr administration (**prevention protocol**). For that, a longitudinal design was performed in two groups of male Wistar-Han rats: I) Normoxic (Nx)+OHTyr group: animals were treated with OHTyr (15 mg/kg in refined olive oil (ROO), by gavage) for 28 days in Nx conditions; II) CIH+OHTyr group: animals were pretreated for 7 days with OHTyr-ROO in Nx conditions, followed by 21 days of CIH conditions and concomitantly OHTyr-ROO treatment. A group of historical controls, exposed to CIH for 21 days, were used as positive control. We observed that OHTyr prevents the increase in SBP induced by CIH, in both active (8am) and inactive (6pm) period of animals, but not DBP.

Encouraged by these results, a **reversion protocol** was performed where rats were exposed to CIH for 21 days and at 22nd day, the OHTyr treatment (15 mg/Kg in vegetable oil, by gavage) was started and maintained for 14 days concomitantly with CIH exposure. We observed that OHTyr administration reverted the increase of SBP and DBP induced by CIH in active period of animals. During inactive period neither SPB nor DBP were decreased with OHTyr administration.

In order to investigate the protective mechanisms of OHTyr, we studied the impact of OHTyr in CIH-modified cysteine dynamics. At the end of reversion protocol kidney cortex and medulla (KC and KM) were collected, in the early phase of inactive period, and total availability of Cys, GSH and CysGly, as well as their reduced and oxidized (free disulfides and protein bound) pools were quantified by HPLC-FD methodology [6]. We observed that 35 days of CIH increased free oxidized Cys fraction at KC and KM and OHTyr treatment was able to revert this increase in both tissues. OHTyr also increased protein cysteinylation in KM. No differences were found in the availability of GSH and CysGly in both KC an KM.

In conclusion, we demonstrated OHTyr as a promising antihypertensive compound in OSA patients. Indeed, its redox actions might renew the focus of antioxidant therapies in OSA-HTN, namely those targeting the non-free radical oxidative species and posttranslational oxidation of proteins.

References: [1] Diogo LN et al. *Eur J Pharmacol.* 2015; 765:58-67; [2] Diogo LN et al. *Arterial Chemoreceptors in Physiology and Pathophysiology*. 2015; 860: 201-209; [3] Psaltopoulou T et al. *Am J Clin Nutr*. 2004; 80(4):1012-8. [4] Moreno-Luna R et al. *Am J Hypertens.* 2012; 25(12):1299-304; [5]; Coelho NR et al. *Adv Exp Med Biol*. 2018; 1071:83-88; [6] Grilo et al. *Eur J Pharm Sci*. 2017; 105:47-54.

Sympathetic Control of Carotid Body Excitability in Spontaneously Hypertensive Rats

Igor S. A. Felippe[1], Tymoteusz Zera[2], Melina P. da Silva[3], Davi J. A. Moraes[3], Fiona McBryde[1], Julian F. R. Paton[1]

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Previous studies have demonstrated that the integrity of the carotid bodies (CBs) is essential for the development and maintenance of hypertension via exacerbated sympathetic drive. CBs of spontaneously hypertensive rats (SHR) exhibit both hypertonicity and hyperreflexia. However, what drives this CB hyper-excitability is not fully understood. We hypothesised that CB hyper-excitability is mediated by its sympathetic innervation, which originates from the superior cervical ganglion (SCG). Experiments were carried out in Wistar and SH rats using the *in situ* working heart-brainstem preparation (WHBP) and *in vivo* telemetric recordings from conscious rats. In the WHBP, phrenic and thoracic sympathetic nerves, heart rate and perfusion pressure were recorded and the chemoreflex activated using NaCN (50-100μL, 0.04%) injected into the aorta. Drug infusions into the CB were made via a cannula placed in the internal carotid artery with its tip juxta-positioned to the carotid body artery. Whole-cell patch-clamp recordings of chemoreceptive petrosal ganglion (PG) neurons were also carried out.

To assess if the SCG modulates the chemoreflex, we stimulated the SCG electrically. This enhanced the chemoreflex evoked sympathoexcitation in both rat strains by 40-50% ($P<0.05$) but was not different between rat strains; there was no obvious modulation in heart rate, perfusion pressure or phrenic nerve responses. The SCG evoked hyperreflexia of the sympathetic chemoreflex was prevented by Tamsulosin, an alpha1-adrenoceptor antagonist, applied to the CB and mimicked by phenylephrine (an alpha1 adrenoceptor agonist); the latter enhanced the chemoreflex sympathoexcitation by 33% ($P<0.05$). Next, we discovered endogenous modulation of the chemoreflex by the SCG in SH rats. Either SCG ganglionectomy (SCGx) or alpha1 adrenoceptor antagonism both reduced the chemoreflex evoked sympathoexcitation ($P<0.01$) to a level equivalent to that observed in Wistar rats. In chemoreceptive PG neurones, SCGx caused hyperpolarization, abolished ongoing firing and reduced the CB evoked firing response; these data were equivalent to those in Wistar rats. In conscious SH rats, the chemoreflex pressor response was attenuated after SCGx, and systolic BP fell by 16 ± 4.85 mmHg. These data demonstrate that the SCG is tonically active in SH (but not Wistar) rats and driving CB hyperexcitability. Immunohistochemistry showed positive co-localisation of alpha1A- and alpha1B- adrenoreceptors on both glomus cells and vessels within the CB. We conclude, sympathetic activity modulates CB reflex sensitivity via alpha1-adrenoreceptors and this appears to be a mechanism underpinning the pathological hyperreflexia of the CB in SH rats. We propose that a positive feedback loop exists in the SH rat whereby CB activity drives the sympathetic nervous system that in turn sensitises the CB and plays a pivotal part in the development of hypertension. Our data support the SCG as a novel target for controlling blood pressure in hypertension.

Support or Funding Information

Health Research Council of New Zealand and Sidney Taylor Trust funded research.

Role of Leptin-TRPM7 Signaling in Carotid bodies in the Pathogenesis of Sleep-Disordered Breathing in Obesity

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Introduction: Sleep-disordered breathing (SDB) affects over 50% of obese individuals. Augmented ventilatory response to hypoxia (HVR) leads to respiratory instability and is one of the cardinal traits of SDB in obesity. Peripheral sensing of hypoxia is mainly governed by the carotid bodies (CB). We have shown that leptin, an adipocyte-produced hormone, acts in the CB to increase ventilation and HVR possibly through the activation of transient receptor potential melastatin 7 (TRPM7) channels. However, the relevance of these findings to diet-induced obese (DIO) mice has not been demonstrated and the effect of leptin-TRPM7 signaling in CB on SDB remains unknown. We hypothesized that leptin acts via TRPM7 in the CB to increase the hypoxic chemoreflex leading to SDB in obesity.

Methods: Male C57BL/6J mice (12 wks-old, n=17) were fed with high fat diet for 8 weeks. DIO mice were headmounted with EEG/EMG electrodes and underwent a full-polysomnography in a whole-body plethysmography chamber. Baseline HVR (10% O_2+3% CO_2 *vs* 20.9% O_2) was performed while awake. Subsequently mice were transfected with *Ad-mCherry-U6-m-Trpm7-shRNA (Trpm7* shRNA, n=9) or control shRNA (n=8) in Matrigel@ administered to the CB area bilaterally. Mice recovered for 9-10 days and HVR/sleep studies were repeated. Another subset of DIO mice (n=5/group) underwent 24-h metabolic studies.

Results: *Trpm7* knockdown in the CB significantly decreased minute ventilation during hypoxia and suppressed HVR during wakefulness compared to baseline (P<0.01) and to control group (P<0.05). *Trpm7* shRNA in the CB increased inspiratory flow, tidal volume and minute ventilation (0.69±0.1 vs 0.81±0.1 mL/min/g) during NREM sleep (P<0.05) with no significant effects on sleep architecture. Consumed O_2, produced CO_2, and respiratory exchange ratio did not change with the *Trpm7* knockdown in the CB.

Conclusions: *Trpm7* knockdown in the CB attenuates the hypoxic chemoreflex during wakefulness and improves the obesity-induced hypoventilation of DIO mice during NREM sleep. Thus, leptin-TRPM7 signaling in the CB could be a potential therapeutic target for the treatment of obesity-related SDB.

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Oxygen Regulation of Breathing is Restored by NDI1 Expression in MCI-Deficient Mice

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Acute O_2 sensing is essential for mammalian homeostasis. The carotid body (CB), the main peripheral chemoreceptor, contains glomus cells that exhibit specific metabolic specializations. In glomus cells, O_2 deficiency (hypoxia) favors the generation of mitochondrial complex I (MCI) signaling molecules, NADH and reactive oxygen species (ROS), which inhibit membrane K^+ channels to induce cell depolarization and transmitter release. Transmitters released during hypoxia activate afferent sensory fibers impinging on the respiratory center to trigger reflex hyperventilation and sympathetic activation (1). Previous experiments on conditional knockout mice lacking Ndufs2, a component of the ubiquinone/rotenone binding site in MCI, have shown complete abolition of systemic hyperventilation and the glomus cell response to acute hypoxia. These observations, demonstrating an essential role of MCI function in acute O_2 sensing, have suggested the mitochondrial to membrane signaling model of chemotransduction in CB glomus cells (2, 3). To further investigate the involvement of MCI in acute O_2 sensing, we have tested whether the expression of an alternative yeast NADH dehydrogenase (NDI1) can restore MCI function in *Ndufs2*-null mice. We have generated a conditional *Ndufs2* knockout mouse model with the transgenic expression of NDI1 protein. Experiments carried out in *Ndufs2-null Ndi1* mice have shown almost complete restoration of the systemic hypoxic ventilatory response. Furthermore, glomus cells from *Ndufs2-null Ndi1* mice show strong rotenone-insensitive secretory responses as well as fast and reversible increases in NADH during exposure to hypoxia. These data indicate that rescue of MCI function by NDI1 is enough to restore responsiveness to hypoxia and provide important insights into the essential role of MCI in acute O_2 sensing. From a more general perspective, our observations demonstrate that heterologous gene therapy can efficiently restore cellular and systemic dysfunctions caused by genetic alterations in MCI-coding genes.

References

1. Fernández-Agüera et al., *Cell Metab*. 2015; 22:825-837.
2. Arias-Mayenco et al., *Cell Metab*. 2018; 28:145-158.
3. Moreno-Domínguez et al. *Sci. Signal.* 2020; 13: eaay9452.

Preliminary Observations of Chemosensitive Thermal Microdomains in Rat Carotid Body Type I Cells

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Detection of a hypoxic stimulus causes closure of potassium channels in carotid body sensory Type I cells and thus initiates cellular depolarization. To date a multitude of hypoxic chemotransduction mechanisms have been described coupling mitochondrial inhibition to potassium channel closure (Rakoczy & Wyatt, 2018). Here, we provide preliminary evidence that support a novel oxygen-sensing hypothesis which couples inhibition of mitochondrial electron transport to a fall in temperature in sub-plasmalemmal microdomains. This fall in temperature may facilitate depolarization in Type I cells.

Distances between antibody-labeled (TOMM20) mitochondria and TASK-potassium channels in rat Type I cells were measured using confocal microscopy. Microdomains were found between mitochondria and TASK-1: 0.33 ± 0.04μm (n = 47) and also TASK-3: 0.32 ± 0.03μm (n = 54). In-vitro experiments using a temperature-sensitive dye (ERthermAC) demonstrated that mitochondrial inhibition by hypoxia caused significant and reversible increases in fluorescence from baseline in isolated Type I cells from 843.3 ± 61.1a.u. to 1044.8 ± 54.3a.u (n = 8, $p<0.0001$) indicating a hypoxia-induced fall in cell temperature; cyanide (2mM) also caused a reversible fall in cell temperature. Finally, whole-cell perforated-patch current-clamp recordings demonstrated that reducing bath temperature from 37°C to 24°C induced reversible depolarizations of 17 ± 2.2mV (n = 5, $p<.01$) in isolated Type I cells.

These data suggest that hypoxic inhibition of mitochondrial thermogenesis may play a role in hypoxic chemotransduction in the carotid body. A consideration of changes in sub-cellular temperature gradients is critical if we are to fully understand how Type I cells and potentially other oxygen-sensitive cells, respond to hypoxia.

RJ Rakoczy is supported by Wright State University's BMS Ph.D. program.

Rakoczy, R. J., & Wyatt, C. N. (2018). Acute oxygen sensing by the carotid body: a rattlebag of molecular mechanisms. *The Journal of Physiology*, *596*(15), 2969-2976.

Beyond the output: the spinal cord's role in hypoxia sensing - Spinal Oxygen Sensors

Nicole O. Barioni[1], Fatemeh Derakhshan[1], Luana Tenorio Lopes[1], Hiroshi Onimaru[2], Arijit Roy[1], Fiona McDonald[1], Erika Scheibli[1], Mufaddal I. Baghdadwala[1], Negar Heidari[1], Manisha Bharadia[1], Keiko Ikeda[3], Itaru Yazawa[4], Yasumasa Okada[3], Michael B. Harris[5], Mathias Dutschmann[6], Richard J.A. Wilson[1].

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BACKGROUND: In hypoxic conditions, cardiorespiratory reflexes are triggered to restore oxygen supply to vital organs including the brain, heart, and kidneys. While the carotid bodies are considered the primary respiratory oxygen chemoreceptors, cardiovascular responses to hypoxia, such as increases in heart rate and blood pressure, persist in their absence. The persistence of cardiovascular responses to hypoxia in the absence of carotid bodies suggest the existence of an additional high fidelity oxygen sensor responsible for eliciting those autonomic reactions. Recently we identified spinal oxygen sensors (SOS) in the intermediolateral cell column of the thoracic spinal cord (spinal preganglionic neurons – SPNs). These neurons are highly oxygen sensitive (excited by hypoxia, silenced by hyperoxia), independent of surrounding astrocytes.

OBJECTIVE: To investigate the functional contribution and cellular mechanism utilized by the SOS during cardiorespiratory crisis.

METHODS: To determine if the SOS are involved in cardiorespiratory responses to asphyxia, we used an *in situ* artificially-perfused rat spinal cord – carotid body - brainstem preparation in which each oxygen sensitive compartment is separately perfused while recording phrenic (respiratory) and splanchnic (sympathetic) nerve activity. To investigate the SOS cellular mechanism of oxygen sensing we recorded from the sympathetic nerve root and also analyzed single cell responses to hypoxia during pharmacological interrogation in artificially-perfused (*in situ*) and slice (*in vitro*) thoracic spinal cord preparations of rats and genetically modified mice.

RESULTS: The SOS can modulate gasp generation by the brainstem and, in some cases, produce gasps on their own. Additionally, the SOS are capable of increasing respiratory and sympathetic activity independent of the brainstem. Furthermore, our results suggest that the SOS are equipped with a novel oxygen sensing mechanism involving neuronal nitric oxide synthase (NOS1), expressed abundantly in SPNs. During normoxia, NOS1 consumes most of the available intracellular oxygen and NADPH. However, during hypoxia, NOS1 becomes dormant due to its high KmO_2, making NADPH available for NOX2. NOX2 launches the production of ROS using the remaining oxygen, which triggers TRP channels (A1 and M4) and IP3R, culminating in elevated intracellular calcium levels and, consequently, neuronal depolarization.

CONCLUSIONS: The results provide novel evidence of an additional life-saving oxygen sensor with a never before realized cellular mechanism of oxygen sensing. This knowledge will assist in therapies for acute and chronic diseases that result in critically low arterial oxygen saturations.