XXth Meeting of the
International Society of Arterial Chemoreception (ISAC)

Making Sense Out of Sensing Hypoxia:
New Directions and Translational Perspectives throughout the Lifespan

CONFERENCE PROGRAM

July 23 - 27, 2017
Charles Commons, Johns Hopkins University
Baltimore, Maryland, USA

http://www.carotidbody.org/
EVENT & SCIENTIFIC ORGANIZING COMMITTEE

Scientific Organizing Committee

Estelle B. Gauda, M.D.
Professor of Pediatrics, University of Toronto
President, ISAC 2014-2017
Director, Toronto Centre for Neonatal Health
Head, Division of Neonatology
Senior Associate Scientist, Research Institute
The Hospital for Sick Children, Toronto, Ontario, Canada

Nanduri Prabhakar, Ph.D., D.Sc
Harold Hines Professor of Medicine
Director, Institute for Integrative Physiology and Center for Systems Biology of Oxygen Sensing
University of Chicago, Chicago, Illinois

Harold Schultz, Ph.D.
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Department of Cellular and Integrative Physiology
University of Nebraska College of Medicine, Nebraska, USA

Chris Wyatt, Ph.D.
Associate Professor of Neuroscience, Cell Biology and Physiology
Wright University, Dayton, Ohio, USA

Event Organizing Committee

Rosie Silva
Sr. Administrative Coordinator for ISAC
Johns Hopkins University, Baltimore, Maryland

Setsuko Takase

Santokh (Sam) Singh

George Kim, M.D.
ISAC Webmaster
Johns Hopkins Hospital, Baltimore, Maryland

Sonia Dos Santos
Administrative Lead
The Hospital for Sick Children, Toronto, Ontario, Canada
Machiko Shirahata Award: Akira Fitzgerald

Robert (Bob) Fitzgerald, Ph.D

We are most grateful for their generous support
Special Note

Publications from the meeting
Keynote and Plenary Talks will be published in a Special Issue of the Journal of Physiology (2017-2018) - Making Sense out of Sensing Hypoxia: New Directions and Translational Perspectives throughout the Life Span
Thank you Harold for submitting the proposal to the J. Physiology.

Proceedings from the meeting
Abstracts will be published by Springer.
The manuscript due date is October 30, 2017. Please mark your calendars.
## 2017 ISAC CONFERENCE PROGRAM

### Sunday, July 23, 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>16:00</td>
<td>Registration - Charles Commons, Johns Hopkins University (3301 N Charles St, Baltimore, MD 21218, USA)</td>
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</tbody>
</table>
| 17:30-18:00 | Welcome Remarks  
E. B. Gauda, President ISAC XX 2017 |
| 18:00  | Opening Reception with Dinner  
• Tribute to Constancio González, M.D., Ph.D. (1949-2015)  
  N. Prabhakar |

### Monday, July 24, 2017

<table>
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<tr>
<th>Time</th>
<th>Activity</th>
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| 7:15-8:10 | Breakfast  
(Posters to be placed on poster boards) |
| 8:15-8:30 | Open Remarks and Housekeeping  
E. B. Gauda |
| 8:30-9:30 | Keynote Speaker:  
Role of the Hypoxia-Inducible Factors in the Carotid Body  
G. Semenza, 2016 Lasker Awardee |

**SESSION 1: Molecular Responses to Hypoxia - New Perspectives**  
*Chair: C. Wyatt*

### ORAL COMMUNICATIONS

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
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</table>
| 9:30-9:45 | Evidence for Dopaminergic Inhibitory Cell-Cell Interactions in the Rat Carotid Body  
  E. M. Leonard, C. A. Nurse |
| 9:45-10:00 | A Novel Index of Steady-State Respiratory Chemoreflex Drive During Incremental Ascent to High Altitude: Relationship to Acute Mountain Sickness  
| 10:00-10:15 | Hypoxia Regulates MicroRNA Expression in the Human Carotid Body  
| 10:15-10:30 | Spinal Oxygen Sensors (SOS) - The Lost Central Cardiovascular Oxygen Sensor?  
  N. Barioni, F. Derakhshan, A. Roy, M. Baghdadwala, F. B. McDonald, E. C. Scheibli, M. B. Harris, M. Dutschmann, R. J. A. Wilson |
| 10:30-11:15 | Coffee Break and Poster Viewing |
### SESSION 2: Central Chemoreception - Oxygen
*Chair: R. Wilson*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>11:15-12:15</td>
<td>Plenary Lecture: Oxygen Sensing in the Central Respiratory Network: Relevance to Disordered Breathing and Cardiorespiratory Control</td>
<td>J. M. Ramirez</td>
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<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>12:15-12:30</td>
<td>CNS Mechanisms of Cardiorespiratory Homeostasis During Hypoxia in Conscious Rats</td>
<td>I. C. Wenker, R. L. Stornetta, P. G. Guyenet</td>
</tr>
<tr>
<td>12:30-12:45</td>
<td>Pro-Inflammatory Cytokines in the Nucleus of the Solitary Tract of Hypertensive Rats Exposed to Chronic Intermittent Hypoxia</td>
<td>M. P. Oyarce, R. Iturriaga</td>
</tr>
<tr>
<td>12:45-13:00</td>
<td>Acute Hypoxia Reveals a Developmentally-Inhibited Rhythm Generator for Lung Breathing in Pre-Metamorphic Tadpole Brainstem Preparations</td>
<td>T. A. Janes, J. P. Rousseau, S. Fournier, R. Kinkead</td>
</tr>
<tr>
<td>13:05-14:15</td>
<td>Lunch - Charles Commons</td>
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### SESSION 3: Hypoxia Sensing in the Carotid Body - New Directions
*Chair: A. Obeso*

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<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>14:15-15:15</td>
<td>Plenary Lecture: Acute Oxygen-Sensing by the Carotid Body: A Rattlebag of Molecular Mechanisms</td>
<td>C. N. Wyatt</td>
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<tbody>
<tr>
<td>15:30-15:45</td>
<td>Molecular Characterization and Localization of Ectonucleotidases in the Rat Carotid Body: Regulation by Chronic Hypoxia</td>
<td>S. Salman, C. Vollmer, G. B. McClelland, C. A. Nurse</td>
</tr>
<tr>
<td>15:45-17:00</td>
<td>Coffee and Poster Viewing</td>
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<tr>
<td>17:15-20:30</td>
<td><strong>BUS PICKUP TIME FROM CHARLES COMMONS IS 17:15</strong> Evening Dinner Group: Crab Feast <strong>BO BROOKS Restaurant, Baltimore, Maryland</strong></td>
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<tr>
<td>7:15-8:10</td>
<td>Breakfast</td>
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<td>8:10-8:15</td>
<td>Housekeeping</td>
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<tr>
<td>8:15-9:15</td>
<td>SESSION 4: (Mal)adaptive Responses to Hypoxia - New Perspectives</td>
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<td>Chair: C. Di Giulio</td>
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<tr>
<td>8:15-9:15</td>
<td>Plenary Lecture: Epigenetics Regulation of Carotid Body Oxygen Sensing: Physiological Consequences from Fetus to Adult</td>
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<td>J. Nanduri</td>
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<td>9:15-9:30</td>
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<tr>
<td>9:15-9:30</td>
<td>Carotid Body Long-Term Facilitation Without CIH Preconditioning: Primarily a Post-Synaptic Mechanism Involving Phosphorylation of TRPV1 and other Post-Synaptic Receptors, Independent of H$_2$O$_2$</td>
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<td></td>
<td>A. Roy, R. J. A. Wilson</td>
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<tr>
<td>9:30-9:45</td>
<td>Contribution of Relative Leptin and Adiponectin Deficiencies to Chronic Intermittent Hypoxia in Premature Infants</td>
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<td>E. B. Gauda, A. Mason, E. Paragon, M. K. Shin, S. Polotsky</td>
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<tr>
<td>9:45-10:00</td>
<td>A New Role of Chronic Intermittent Hypoxia on Pathology: Spontaneous Tumorigenesis and Consequences</td>
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<td>M. T. Gallego-Martin, E. Gonzalez-Obeso, E. Olea, I. Docio, A Rocher, A. Obeso</td>
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<tr>
<td>10:00-10:30</td>
<td>Coffee Break and Poster Viewing</td>
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<td>10:30-11:30</td>
<td>SESSION 5: Role of Chemoreception in Disease - New Perspectives</td>
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<td>Chair: J. Kåhlin</td>
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<tr>
<td>10:30-11:30</td>
<td>Plenary Lecture: Translating Carotid Body Function in Clinical Medicine</td>
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<td></td>
<td>R. Iturriaga</td>
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<tr>
<td>11:30-11:45</td>
<td>ORAL COMMUNICATIONS</td>
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<tr>
<td>11:30-11:45</td>
<td>Lysophosphatidic Acid Stimulates the Carotid Body: Linking Inflammation and Carotid Sinus Nerve Activity</td>
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<td></td>
<td>N. G. Jendzjowsky, A. Roy, R. J. A. Wilson</td>
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<tr>
<td>11:45-12:00</td>
<td>Carotid Sinus Nerve Resection Decreases Weight Gain and Fat Mass in a Rodent Model of Obesity</td>
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<tr>
<td>12:00-12:15</td>
<td>Overactivation of Carotid Sinus Nerve Activity in High-Fat Animals is Mediated by A$_2$ Adenosine Receptors</td>
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<td>J. F. Sacramento, J. Prieto-Lloret, S. V. Conde</td>
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<tr>
<td>12:15-12:30</td>
<td>Therapeutic Targeting of Carotid Body for Treating Sleep Apnea in a Pre-Clinical Model</td>
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<tr>
<td>12:30-13:45</td>
<td>Lunch</td>
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### SESSION 6: Role of Chemoreception in Disease - New Directions

**Chair:** S. Conde

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>13:45-14:45</td>
<td>Plenary Lecture: What Else Do the Carotid Bodies Do? M. Joyner</td>
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<th>Time</th>
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<tbody>
<tr>
<td>14:30-14:45</td>
<td>Contrasting Effects of Acute and Chronic Progesterone Administration on Apnea Frequency in Newborn Male and Female Rats N. P. Uppari, H. Kouchi, V. Joseph, A. Bairam</td>
</tr>
</tbody>
</table>

#### Wednesday, July 26, 2017

- **9:30-20:30 Site Seeing Group Activity (Washington DC and Dinner)**
  - 9:30 - Meet at Bus: Charles Commons
  - To Washington - Mall, Museums, Galleries (free admission)
  - 17:30 - Meet at Bus in DC
  - 18:15-20:30 - Sequoia Restaurant on the Potomac River, Washington, DC (Happy Hour followed by Dinner)

#### Thursday, July 27, 2017

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>8:15-9:00</td>
<td>Breakfast</td>
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### SESSION 7: Role of Chemoreception in Disease - Early Development

**Chair:** A. Bairam

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>9:00-10:00</td>
<td>Plenary Lecture: Peripheral and Central Chemoreceptors in Sudden Infant Death Syndrome A. Porzionato</td>
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<th>Time</th>
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<tbody>
<tr>
<td>10:00-10:15</td>
<td>Divergent Effects of Myo-Inositol on Respiratory Neural Control During Postnatal Development P. M. MacFarlane, J. M. Di Fiore, R. J. Martin, A. Mayer, I. R. Bederman</td>
</tr>
<tr>
<td>10:15-10:30</td>
<td>Protection from SIDS: Perhaps it is in the Milk E. B. Gauda</td>
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<tr>
<td>10:30-11:15</td>
<td>Coffee and Poster Viewing</td>
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</table>
### SESSION 8: Role of Chemoreception in Disease - Aging  
*Chair: H. Schultz*

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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</table>
| 11:15-12:15 | Plenary Lecture: The Aging Carotid Body  
*C. Di Giulio*                                      |
| 12:15-13:30 | Lunch and Poster Viewing                                                              |

### SESSION 9: Central Chemoreception - Carbon Dioxide  
*Chair: E. Monteiro*

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<th>Time</th>
<th>Event</th>
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</table>
| 13:30-14:30 | Plenary Lecture: Homeostatic Regulation of Arterial PCO₂ by the Retrotrapezoid Nucleus (RTN): Neural Circuits and Molecular Mechanisms of CO₂ Sensing  
*P. Guyenet*                                      |

### SESSION 10: Molecular Responses to Hypercapnia - New Directions  
*Chair: R. Iturriaga*

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<tr>
<th>Time</th>
<th>Event</th>
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</table>
| 14:45-15:45 | Plenary Lecture: Regulation of Inflammatory Gene Expression by Hypoxia and Hypercapnia  
*C. Taylor*                                      |
| 16:00-16:10 | Announcements by E. B. Gauda                                                              |
| 16:10-17:30 | Free Time                                                                              |
| 17:30    | Coach Bus - Leaves from Charles Commons to Peabody Library for GALA                     |
| 18:00-18:30 | Pre-Dinner Reception                                                                    |
| 18:30-20:30 | GALA – Peabody Library, Johns Hopkins University                                      |
|          | **Tribute to Machiko Shirahata, M.D. (1950-2016)**                                      |
|          | *J. Sham*                                                                               |
|          | **Machiko Shirahata ISAC Trainee Travel Awardees:**                                     |
|          | **Recognition of Awardees by Estelle Gauda, M.D. and Seva Polostky, M.D., Ph.D.**       |
|          | **De Castro Awards – Presentation of Certificates**                                      |
|          | **Recognition of Awardees by Ana Obeso**                                                |
| 21:00    | Board Bus to Return from GALA                                                           |
PLEASE NOTE: Many talks will also be presented as posters. The list below represents only abstracts that will be presented as posters. Please can the numbered poster boards be left for the posters (1 through 15) noted below. Any other free poster boards are available for use by those who are providing posters as well as talks. All posters can remain on display for the duration of the meeting.

P1. Hypoxia-Induced Rise in $[Ca^{2+}]_i$ in Rat Carotid Body Glomus Cells: Does it Involve Inhibition or Activation of BK/Kv?
   J. Wang, D. Kim

P2. Expression and Function of TASK-1 and TASK-3 in Rabbit Carotid Body Glomus Cells
   D. W. Kang, J. Wang, J. O. Hogan, D. Kim

P3. Molecular and Functional Analysis of T-Type Ca$^{2+}$ Channels in Rat Carotid Body

P4. Mitochondrial Complex I Dysfunction and Oxygen Sensing in a FASTK-Deficient Mice Model

P5. Topical Application of Connexin-Based Hemichannel Blocker Reduces Carotid Body-Mediated Chemoreflex Drive in Rats
   D. C. Andrade, R. Iturriaga, C. Toledo, C. M. Lucero, H. S. Diaz, A. Arce-Álvarez, M. Retamal, R. Del Río

P6. Guinea Pig as a Negative Model to Study the Carotid Body Mediated Chronic Intermittent Hypoxia Effects

P7. Lactate Sensing by Carotid Body Glomus Cells
   H. Torres-Torrelo, I. Arias-Mayenco, P. Ortega-Sáenz, J. López-Barneo

P8. Acute Effects of Systemic Erythropoietin Injections on Carotid Body Chemosensory Activity Following hypoxic and Hypercapnic Stimulation
   R. Iturriaga, D. C. Andrade, F. Jeton, N. Voituron, R. Del Río

P9. Leptin in the commissural nucleus tractus solitarii increases the glucose responses to carotid chemoreceptors activation by cyanide
   M. Lemus, C. Mojarror, S. Montero, V. Melnikov, M. Ramírez-Flores, E. Roces de Álvarez-Buylla

P10. High Fat Feeding in Rats Alters Respiratory Parameters by a Mechanism that is Unlikely to be Mediated by Carotid Body Type I Cells
    R. Rakoczy, R. L. Pye, T. H. Fayyad, J. M. Santin, B. L. Barr, C. N. Wyatt

P11. Increased Day/Night Changes in Hypoxic Ventilatory Response in Chronic Heart Failure
     R. Lewis, B. T. Hackfort, H. D. Schultz

P12. Effect of Chronic Hypoxia on Pulmonary Hypertension and Endothelial Function in the Rat
     Prieto-Lloret, I. Docio, E. Olea, A. Gomez-Niño, A. Rocher

P13. Sexual Dimorphism on the Effects of Chronic Hypoxia in Rat

P14. Chronic Heart Failure Abolishes Circadian Rhythms in Chemoreflex Breathing
     R. Lewis, B. T. Hackfort, H. D. Schultz

P15. Focusing on a Non-Invasive Measurement of Sympathetic Reactivity to Central and Peripheral Chemoreceptor Stimulation in Humans
     N. G. Jendzjowsky, C. D. Steinback, R. J. Herman, W. H. Tsai, F. E. Costello, R. J. A. Wilson
Oxygen Sensing in the Central Respiratory Network: Relevance to Disordered Breathing and Cardiorespiratory Control

Jan Marino (Nino) Ramirez, Ph.D.

Director, Center for Integrative Brain Research, Seattle Children's Research Institute, USA

Departments of Neurological Surgery and Pediatrics, University of Washington School of Medicine, Seattle USA

All mammals developed effective strategies to cope with reduced oxygen availability or other metabolic, environmental and behavioral challenges. An important prerequisite for survival is the necessity to maintain functional integrity during times of extreme challenges. This is particularly important for the neuronal control of breathing and the cardiovascular system. Mammals adapt to hypoxia through a highly coordinated, systems- and cellular level reconfiguration involving the partial shutdown of some but not all organs. This reconfiguration is controlled through a similarly complex reconfiguration at the cellular and network level within the central nervous system. Hypoxia and reactive oxygen species significantly alter the composition and even distribution of respiratory activity within the ventral respiratory column. This network reconfiguration, involving the differential activation and inhibition of respiratory neurons as well as glia, is initially adaptive but becomes maladaptive following exposure to chronic intermittent hypoxia leading to disordered breathing and disturbed cardiorespiratory coupling.
Acute Oxygen-Sensing by the Carotid Body: A Rattlebag of Molecular Mechanisms

Chris N. Wyatt, Ph.D.

Associate Professor of Neuroscience, Cell Biology and Physiology, Wright University, Dayton, Ohio, USA
Wright State University, Dayton, Ohio

The following topics will be discussed:

The molecular underpinnings of the oxygen-sensitivity of the carotid body type I cells are becoming better and better defined as research begins to integrate independent mechanisms into functional models. Nevertheless, the field of oxygen-chemoreception still presents the general observer with a bewildering array of potential signaling pathways by which a fall in oxygen levels might initiate type I cell activation. The purpose of this brief lecture is to address five of the current oxygen-sensing hypotheses.

1. The AMP-activated protein kinase hypothesis will be defined and its current role in oxygen-sensing by the carotid body, and hypoxic chemotransduction in general discussed
2. The relatively new lactate/Olfr78 receptor hypothesis of oxygen chemotransduction at the level of the type I cell will be presented and critiqued.
3. The current status of the mitochondrial hypothesis and the role ATP production may have in chemotransduction will be presented.
4. How reactive oxygen species may present themselves as key transducers in the oxygen-sensing cascade will be outlined.
5. Finally the mechanisms by which H2S, reactive oxygen species and Heme Oxygenase may integrate to provide a rapid oxygen-sensing transduction system will be addressed.

Several of these chemotransduction mechanisms share similar themes and an attempt to integrate them in the context of the unique subcellular structure of the type I cells will be made. An emphasis will be put on the subplasmalemmal-mitochondrial microenvironment in type I cells and how that is likely to play a critical role in any proposed oxygen-sensing mechanism.
Epigenetic regulation of Carotid body Oxygen Sensing: Physiological Consequences from Fetus to Adult

Jayasri Nanduri, Ph.D.

Associate Professor, Institute for Integrative Physiology and Center for Systems Biology of O₂ Sensing, Biological Science Division, University of Chicago, Chicago, IL

The carotid bodies are the principal sensory organs for detecting arterial blood oxygen levels, and the resulting chemoreflex is a potent regulator of the sympathetic tone, blood pressure and breathing. Sleep apnoea is a disorder of respiratory system affecting several million adults and infants born preterm. Patients with sleep apnoea exhibit several co-morbidities, including heightened sympathetic nerve activity and hypertension. Emerging evidence suggests that intermittent hypoxia (IH) resulting from periodic apnoea stimulates the carotid body, and the ensuing chemoreflex mediates the increased sympathetic tone and hypertension in individuals with sleep apnoea. Exposure of neonatal rat pups to IH (simulating the O₂ saturation profiles encountered during sleep apnoea) or adult rats to long-term IH (30d) lead to persistent activation of the chemoreflex, and elevated ROS levels, which were attributable in part to markedly reduced expression of antioxidant enzyme (AOE) genes in the chemoreflex pathway. AOE genes that are downregulated by long-term and neonatal IH display DNA hypermethylation and increased DNA methyl transferase (Dnmt) enzyme activity in the chemoreflex pathway, and these effects are blocked by treating rats with decitabine, a DNA hypomethylating agent, along with normalization of blood pressures and breathing (Nanduri et al., 2012; Nanduri et al., 2017). Selective CB ablation prevented LT-IH elevated ROS levels in the adrenal medulla, nucleus tractus solitarius and rostral ventrolateral medulla, representing the peripheral and central components of the CB chemo-reflex, and this effect was associated with absence of transcriptional repression of AOE genes by DNA methylation as well as increased Dnmt protein abundance and enzyme activity. Our data further showed that CB neural activity dependent ROS generation increases DNA methylation through increased expression of Dnmt proteins as a result of Akt-dependent inactivation of GSK3β. These findings demonstrate a hitherto uncharacterized CB neural activity-dependent epigenetic regulation of redox state in response to IH.
Translating Carotid Body Function in Clinical Medicine

Rodrigo Iturriaga, M.D.

Laboratorio de Neurobiología, Pontificia Universidad Católica de Chile Santiago, Chile

The classical physiological paradigm considers the carotid body (CB) as the main peripheral oxygen sensor, whose afferent sensory discharge regulates the reflex adjustments to maintain the blood respiratory gases and pH homeostasis. Accordingly, the CB mediates the fast cardio-respiratory chemoreflex responses to acute hypoxemia and the ventilatory acclimatization to chronic hypoxemia at high altitude. In clinical medicine, the main pathological disease associated with the CB are the chemodectomas, which are highly vascular glomus tumors. However, a growing body of new experimental evidence supports the novel idea that an abnormal enhanced CB chemosensory discharge contributes to the sympathetic overactivation. Indeed, the CB has been implicated in several diseases associated with increases in sympathetic outflow, including severe hypertension, heart failure, obstructive sleep apnea (OSA), and metabolic syndrome. The mechanisms underlaying the potentiation of the CB chemosensory discharge elicited by these diseases are not completely known. However, it is likely that ROS-dependent alterations of chemosensory transmitters, modulators and Ca2+ signalling, are involved in the neural sensory potentiation. Indeed, chronic intermittent hypoxia (CIH), the main feature of OSA, produces CB oxidative stress, which is associated with reduced NO, and increases ET-1, angiotensin II and pro-inflammatory cytokines levels in the CB. Furthermore, CIH alters the balance of the hypoxia-inducible factors HIF-1α/HIF-2α favoring the transcription of pro-oxidant enzymes, and leading oxidative stress. Similarly, oxidative stress, angiotensin II and reduction of NO levels have been related to the augmented CB discharge induced by heart failure. In addition, the CB has been proposed as the systemic metabolic sensor of insulin levels. Hypercaloric diets produces CB potentiation, which contributes to autonomic alterations, insulin resistance and hypertension. The ablation of the CBs or the carotid sinus neurotomy abolished the autonomic alterations induced by CIH, heart failure and hypercaloric diets. Moreover, the ablation of one CB has been proposed for the clinical treatment of resistant hypertension in humans. I will analyze and discuss new evidence supporting a novel role for the CB chemoreceptor in the progression of autonomic, cardiorespiratory and neuro-hormonal alterations induced by CIH, heart failure and metabolic diseases.

Work supported by FONDECYT 1150040

Authors confirm that the experiments described here have received ethical approval.
What Else Do the Carotid Bodies Do?

Michael J. Joyner, M.D.

Frank R. and Shari Caywood Professor of Anesthesiology, Dept. of Anesthesiology & Perioperative Medicine, Mayo Clinic Rochester, Minnesota, USA

In this talk I will review emerging evidence from animal models and human studies on the emerging idea that the carotid bodies (CBs) play a key role in the regulation of blood pressure and also metabolism. A key question is the extent to which the CBs contribute to baseline regulation of sympathetic nerve activity and gain in response to sympathoexcitatory stimuli vs. their potential role as metabolic sensors per se that initiate physiological responses. Issues surrounding the targeting of the CBs for therapeutic purposes will also be discussed.
Peripheral and Central Chemoreceptors in Sudden Infant Death Syndrome

Andrea Porzionato, Ph.D.*[1], Veronica Macchi [1] and Raffaele De Caro [1]

*Associate Professor of Human Anatomy, Department of Human Anatomy and Physiology

Institute of Human Anatomy, Department of Neuroscience, University of Padova [1]

According to the 'classical' triple-risk model, the pathogenesis of Sudden Infant Death Syndrome (SIDS) has been referred to an underlying biologic vulnerability to exogenous stressors or triggering factors in a critical developmental period. Clinical and histopathological findings in premature newborns and SIDS victims, together with experimental data from animal models, indicate the possible involvement of nervous structures directly or indirectly involved in peripheral (carotid and aortic bodies, neuroepithelial bodies) and central (retrotrapezoid nucleus/parafacial respiratory group, solitary tract nucleus, medullary raphe nuclei, locus coeruleus and others) chemoreception. Peripheral/central chemoreceptors undergo critical maturation in the perinatal period, with progressive increase in chemosensitivity. These structural and functional changes may be negatively affected by prematurity and by a series of environmental factors (exposure to tobacco smoke, substances of abuse, hyperoxia and continuous or intermittent hypoxia) which are known to increase the risk of SIDS. In SIDS victims, cytochemical and morphometric alterations have also been reported in peripheral and central chemoreceptors, supporting a biologic vulnerability due to immature or maladaptative chemoreceptive mechanisms.
The aging process is characterized by a decline in several physiological functions resulting in a reduced capability to maintain homeostasis. This lowered homeostatic capacity seems to involve the carotid body (CB), whose role is to modulate ventilation and tissue oxygen supply thus playing a prime role in all aging processes. Aging causes marked changes in CB morphology. Indeed, it is enlarged and shows a concomitant decrease in the percentage of chemoreceptor tissue, as well as a proliferation of Type II cells. The carotid glomus is present with aggregates of lymphocytes and fibrosis of the lobules. Type I cells are dehydrated, with a profound vacuolization, a shrinking nucleus, and lipofuscin accumulation. With increased age man CB shows a reduction in the number and volume of mitochondria, fewer synaptic junctions between glomus, along with a reduction in CB content of neurotransmitters, leading to a sort of ‘physiological denervation’. Instead, in rats the hyperplastic response of CB cells during chronic hypoxia is less evident in aged CB samples as compared to young ones. The increase in HIF-1 - VEGF - ET and NOS-1 expression during chronic hypoxia is less evident in CBs of old rats as compared to the young ones. This favors changes in the set-point sensitivity for the chemosensory peripheral drive. Aging could be interpreted as a cumulative result of oxidative damage to cells, which derives from aerobic metabolism. Moreover, metabolism rate is tightly correlated with life span, thus a loss in mitochondrial function is one of the prime factors affecting CB aging processes. The age-related reduction in synaptic junctions might be a self-protective mechanism through which cells buffer themselves against accumulation of reactive oxygen species during aging. The correlation between hypoxia and life-span of CB cells remains open until the question of how and why cells sense oxygen is solved. In other words, in order to better understand aging, knowledge of which O$_2$ species are being sensed by cells is needed.

The author confirm that the experiments described here have received ethical approval.
Homeostatic Regulation of Arterial PCO₂ by the Retrotrapezoid Nucleus (RTN): Neural Circuits and Molecular Mechanisms of CO₂ Sensing

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The following topics will be covered:

Defining the RTN: brief overview of the transmitters, receptors, developmental lineage and axonal projections of the RTN.

Three classes of mechanisms may underlie the exquisite sensitivity of RTN neurons to changes in arterial PCO₂ in vivo (0.5Hz / 0.01pHa): intrinsic proton sensitivity of RTN neurons mediated by a potassium channel, TASK-2, and a GPCR (GPR4), astrocyte-dependent paracrine mechanisms regulating local blood flow and tissue pH, and synaptic inputs from other CO₂-activated neurons.

RTN neurons stimulate lung ventilation by regulating multiple aspects of breathing (frequency, inspiratory amplitude, post-inspiratory brake and active expiration). RTN neurons drive breathing in direct proportion to arterial pH below a threshold pH 7.5.

The carotid bodies stimulate breathing by activating RTN and via unidentified pathways that bypass this nucleus. This network configuration is ideally suited for vigorous breathing stimulation by hypercapnic (or acidotic) hypoxia. However, pure hypoxia (<12% FiO₂) silences RTN via respiratory alkalosis thereby opposing the breathing stimulus elicited by the carotid bodies. This phenomenon probably explains why acute poikilocapnic hypoxia is such a poor respiratory stimulus in rodents compared to hypercapnia.

Breathing control by RTN is state-dependent. Most notably, RTN regulates breathing frequency only when the central respiratory pattern generator is auto-rhythmic (anesthesia, non-REM, quiet waking). This peculiarity accounts for the reduced influence of RTN on breathing during REM sleep and may explain the much lower incidence of central sleep apnea and periodic breathing during this sleep stage in humans.
Regulation of Inflammatory Gene Expression by Hypoxia and Hypercapnia

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Professor, School of Medicine, University College Dublin, Ireland

1UCD Conway Institute, Systems Biology Ireland and School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland. Immunological niches are focal sites of immune activity that can have varied and context-dependent micro-environmental features. Hypoxia and hypercapnia are features that are commonly associated with both physiologic and pathophysiologic states in which immunological niches play a central role. Depending upon the cell types present, the microenvironment and the immune processes occurring in a given niche, the impact of hypoxia or hypercapnia on immunity and inflammation can vary greatly. Here we discuss the effects of hypoxia and hypercapnia on immune cells and the consequences of these distinct stimuli for immunity and inflammation in different immunological niches.
ORAL COMMUNICATIONS
Evidence for Dopaminergic Inhibitory Cell-Cell Interactions in the Rat Carotid Body


McMaster University, Department of Biology, Hamilton, Ontario, Canada [1]

Mammalian carotid bodies (CB) are the main peripheral arterial $\text{O}_2$ and $\text{CO}_2/\text{H}^+$ chemoreceptors, strategically located at the bifurcation of the common carotid artery. Purinergic signaling pathways contribute to the increased afferent discharge during sensory transduction. For example, ATP, released from chemoreceptor (type I) cells, may stimulate ionotropic P2X2/3 and metabotropic P2Y2 receptors (P2Y2R) on adjacent afferent nerve endings and glial-like type II cells, respectively. Paracrine stimulation of P2Y2R on type II cells may lead to the further release of ATP via pannexin-1 channels, thereby boosting excitation. Using ratiometric calcium imaging, we investigated the potential of dopamine (DA), a monoamine synthesized by type I cells, to modulate communication between type I and type II cells. When applied alone, DA (10 µM) evoked negligible intracellular Ca$^{2+}$ responses in type II cells. By contrast, DA attenuated P2Y2R-mediated Ca$^{2+}$ signaling in 75-100% of type II cells. This inhibitory action of DA was prevented by the D2 receptor blocker, sulpiride. In addition, sulpiride potentiated intracellular Ca$^{2+}$ responses in type II cells during stimulation of type I cells by hypercapnia (10% CO$_2$). We hypothesize that paracrine DA inhibitory pathways may help regulate CB excitation by modulating purinergic signaling.

We wish to thank NSERC and CIHR for funding.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
A Novel Index of Steady-State Respiratory Chemoreflex Drive During Incremental Ascent to High Altitude: Relationship to Acute Mountain Sickness

Pfoh JR [1], Bruce CD [1], Vanden Berg ER [1], de Freitas EM [1], Borle K [1], Lavoie LR [1], Saran G [1], Linares A [1], Brandt R [1], Tjandra K [1] and Day TA [1]

Department of Biology, Faculty of Science and Technology, Mount Royal University, Calgary, AB, Canada [1]

The measurements of central and peripheral respiratory chemoreflexes are important in the context of high altitude as indices of ventilatory acclimatization. However, respiratory chemoreflex tests have many caveats in the field, including considerations of safety, portability and consistency. We recently characterized a novel index of steady-state respiratory chemoreflex drive (SS-CD) during normobaric hypoxia (Pfoh et al., 2017). The SS-CD takes into account the contribution of central and peripheral respiratory chemoreceptors, and eliminates the need for transient respiratory gas perturbation tests. First, steady-state measurements of the pressure of end-tidal (P\textsubscript{ET}) CO\textsubscript{2} (Torr) and peripheral oxygen saturation (SpO\textsubscript{2}; %) are used to quantify a stimulus index (SI; P\textsubscript{ET}CO\textsubscript{2}/SpO\textsubscript{2}). The SS-CD is then calculated by indexing resting ventilation (V\textsubscript{I}; L/min) against the SI. We aimed to characterize the SS-CD in the context of incremental high altitude ascent in the Nepal Himalaya in 11 healthy lowlander participants using prophylactic Diamox (26.2±7.4 yrs, BMI 23.8±3.9 kg/m\textsuperscript{2}, 8 females). Measurements were made at 1045/1400m, 3440m, 3860m and 4370m including resting V\textsubscript{I} (calibrated pneumotachometer), P\textsubscript{ET}CO\textsubscript{2} (portable capnography), SpO\textsubscript{2} (portable pulse oximeter), urine pH (portable calibrated pH meter) and self-reported acute mountain sickness (AMS) scores. Participants further ascended to above 5000m for additional AMS scoring. As expected, V\textsubscript{I} increased with ascent (P=0.001), both P\textsubscript{ET}CO\textsubscript{2} and SpO\textsubscript{2} decreased (P<0.001), urine pH increased (P=0.005), and the SS-CD increased (P<0.0001), indicating that participants acclimatized from both renal and ventilatory perspectives. The SS-CD at 4370m was negatively correlated (p=-0.63, P=0.04) with AMS scores after one night at a 4910m, suggesting that the SS-CD magnitude may have some relationship to protection against AMS severity. This novel SS-CD may have future utility in assessing ventilatory acclimatization during prolonged stays at altitude, and may replace the use of complex and confounded transient peak response tests of the hypoxic ventilatory response at high altitude. (297 words)


Funding and Ethics: Funding was provided by (a) Alberta Innovates Health Solutions Summer Studentship, (b) Natural Sciences and Engineering Research Council (NSERC) Undergraduate Student Research Assistantship, (b) Alberta Government Student Temporary Employment Program, and NSERC Discovery Grant program. The authors confirm that the experiments described here have received ethical approval (MRU Human Research Ethics Board 2015-26b, Nepal Health Research Council 96/2015).
Hypoxia Regulates MicroRNA Expression in the Human Carotid Body

S. Mkrtchian [1], K. L. Lee [2], J. Kåhlin [1, 3], A. Ebberyd [1], L. Poellinger [2, 4], M. Jonsson Fagerlund [1, 3] and L. I. Eriksson [1, 3]

Section for Anesthesiology and Intensive Care Medicine, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden [1], Cancer Science Institute of Singapore, National University of Singapore, Singapore [2], Function Perioperative Medicine and Intensive Care, Karolinska University Hospital, Stockholm, Sweden [3], Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden, [4]

The mechanisms of hypoxia-mediated regulation of gene expression in the human carotid body (CB) remain poorly understood. While limited information on transcriptional regulation in animal CBs is available, the identity and impact of important post-transcriptional regulators such as non-coding RNAs, and in particular miRNAs are not known. Here we show that the acute hypoxic conditions applied to the surgically removed human CB slices lead to the differential regulation of a number of miRNAs. These hypoxia-regulated miRNAs target biological pathways with upregulation of functions related to cell proliferation and immune response and downregulation of cell differentiation and cell death functions. Upregulation of proliferative processes may be mediated by the key hypoxia-inducible factor HIF-1α that is targeted by the HIF-1α specific miR-18b, which was downregulated during hypoxia in the human CB. Upregulation of yet another hypoxia affected miRNA, miR-155 may be one of the factors involved in the hypoxia-mediated release of pro-inflammatory cytokines including TNFα as described in our previous report (Kåhlin et al., 2015).

Comparative analysis of the human CB miRNAome with the global miRNA expression patterns of a large number of different human tissues showed that the CB miRNAome had a unique profile, which reflects its highly specialized functional status. Nevertheless, the human CB miRNAome is most closely related to the miRNA expression pattern of brain tissues indicating they may have a common developmental origin.


This research was supported by research grants from the Research Council for Medicine, Sweden, Stockholm County Council, Thorsten Söderberg Research Foundation, Gösta Fraenckels Foundation, Jeanssons Foundation, Tore Nilsons Foundation, Magnus Bergvalls Foundation, Capio Foundation, LPS Medical, Karolinska Institutet Funds and The Swedish Society for Medicine, all from Stockholm, Sweden.

Experimental protocols were approved by the ethics committee on human research at the Karolinska Institutet in Stockholm, Sweden and in agreement with the Declaration of Helsinki.
Spinal Oxygen Sensors (SOS) – The Lost Central Cardiovascular Oxygen Sensor?

N. Barioni [1], F. Derakhshan [1], A. Roy [1], M. Baghdadvala [1], F. B. McDonald [1], E. C. Scheibli [1], M. B. Harris [2], M. Dutschmann [1], R. J. A. Wilson [1]

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BACKGROUND: The carotid bodies are archetypal respiratory oxygen chemoreceptors. However, several studies have reveal a persistent cardiovascular (sympathetic) response to hypoxia sans arterial chemoreceptors, suggesting another important -- likely central -- oxygen sensor. Here we report an exquisitely sensitive oxygen sensor in the spinal cord and describe our recent work to understand its properties.

METHODS: All experiments were performed in either an anesthetized, vagotomized, artificially-ventilated in vivo preparation with carotid body and aortic arch denervated, or a novel artificially-perfused in situ thoracic spinal cord preparation (no confounding brainstem, blood or endocrine influences, while allowing direct experimental control of spinal cord PO$_2$ and PCO$_2$). Sympathetic nerve activity (SNA) was used as the output variable and analyzed using automated software.

RESULTS: SNA responses to slight (5%) increases or decreases in oxygen saturation in vivo persisted following spinalization (no brainstem). In the artificially-perfused thoracic spinal cord preparation, the sensitivity of SNA in the hyperoxic range exceeded that of an equivalent artificially-perfused en bloc carotid body preparation. Oxygen sensitivity was present by postnatal day 3 (P3), and could be mimicked by NaCN but not CO$_2$. Oxygen sensitivity persisted with glutamatergic and purinergic receptor blockers, but was abrogated by calcium channel blockers. Reducing spinal perfusion pressure also increases SNA. Preliminary data suggest that in the presence of TTX (used to block spike-mediated neuronal interactions), reductions in spinal perfusion induces cFos in preganglionic sympathetic neurons located in the intermediolateral (IML) cell column of the spinal cord.

CONCLUSIONS: These data suggest that preganglionic sympathetic neurons constitute highly responsive oxygen and perfusion sensors, that are present soon after birth and capable of directly regulating cardiovascular function. Interestingly, other investigators report the IML is abnormal in a large proportion of SIDS cases, suggesting that oxygen-sensing preganglionic neurons maybe critical for neonatal survival.

R.J.A.W. is an Alberta Innovates Senior Scholar and N.B is supported by a University of Calgary Eyes High Recruitment Studentship. Funded for this project is provided by CIHR (R.J.A.W).

Where applicable, the authors confirm that the experiments described here have received ethical approval.
CNS Mechanisms of Cardiorespiratory Homeostasis During Hypoxia in Conscious Rats


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In mammals, systemic hypoxia produces a number of physiological responses and, if chronic or repeated, may contribute to certain pathologies, such as hypertension. Here we explore the contribution of the C1 neurons to cardiovascular homeostasis during hypoxia. C1 neurons were transduced to express Archaerhodopsion using a lentiviral vector, so that optogenetic photoinhibition could be achieved in conscious, freely behaving rats.

Although hypoxia produces vasodilation, mean arterial pressure (MAP) and heart rate (HR) are only minimally affected. As we have demonstrated previously, photoinhibition of C1 neurons produces hypotension and bradycardia (Figure 1, black), indicating the C1 neuronal activity contributes to cardiovascular homeostasis during hypoxia. The hypotension elicited by C1 inhibition was blocked by prazosin (Figure 1, green), an α-adrenergic antagonist. Surprisingly, the bradycardia was blocked by atropine (Figure 1, red), but not by propranolol (data not shown). Thus, it would appear that the C1 neurons stimulate the sympathetic system to maintain blood pressure, but actively inhibit the parasympathetic system to maintain heart rate.

We also observed evidence for CNS control of sighing during hypoxia. Interestingly, hypoxia-evoked sighs are blocked by propranolol, but not by sotalol (Figure 2, blue and red trace, respectively). Both are non-specific β-adrenergic receptor antagonists, however only propranolol crosses the blood brain barrier, suggesting central β receptors are crucial for hypoxia induced sighing, as has been suggested before. We propose that hypoxia activates C1 and other catecholaminergic neurons, which stimulate β receptors in respiratory control centers to produce sighs.

This work was supported by grants from the National Institutes of Health (RO1 HL074011 and RO1 HL028785 to PGG and F32HL127975 to ICW).

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Abstract No. 6

Pro-Inflammatory Cytokines in the Nucleus of the Solitary Tract of Hypertensive Rats Exposed to Chronic Intermittent Hypoxia

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Obstructive sleep apnea is characterized by chronic intermittent hypoxia (CIH), which is considered the main risk factor for developing hypertension. Pro-inflammatory molecules had been involved in neurogenic hypertension models acting on brain cardiovascular regions like the nucleus of tractus solitarius (NTS), the primary site for carotid body (CB) afferent inputs, the main peripheral chemoreceptor. 14 days of CIH exposure of rats augmented the expression of pro-inflammatory cytokines (PIC) in the CB (Del Rio et al., 2012). We aim to study if PIC in the NTS play a role in the development of the hypertension induced by CIH-treatment. Previously we showed that rats become hypertensive at 7 days of CIH exposure (89.3 ± 2.5 mmHg vs 116.9 ± 13.2 mmHg, p < 0.05 sham vs CIH, n=4). Male Sprague-Dawley rats 250g were exposed to CIH (5% O2, 12 times/h, 8 h/day) for 7, 14 or 28 days. The rats were euthanized and brains were removed and processed to assess IL1-β, IL-6 and TNF-α in the NTS using immunofluorescence. No significance changes in PIC immunoreactivities levels were found at 7 or 14 days of CIH exposure respect Control rats (n=3). A significant increase of PIC immunoreactivities levels was observed within the NTS at day 28 of CIH exposure compared with Control (1629.0 ± 160.1 vs 879.3± 189.6 au, IL1-β; 1342 .0 ± 75.9 vs 863.3± 186.1 au, IL-6 and 723.2 ± 67.0 vs 293.1± 48.3 au, TNF-α; * p <0,01 ANOVA one way followed by Dunnett’s test). PIC immunofluorescence was observed on cytoplasm and nucleus of cells. Present results suggest that pro-inflammatory cytokines actions in the NTS could be one of the possible mediators in the maintenance of hypertension in CIH-exposed animals.


Work supported by FONDECYT 1150040

Authors confirm that the experiments described here have received ethical approval.
Abstract No. 7

Acute Hypoxia Reveals a Developmentally-Inhibited Rhythm Generator for Lung Breathing in Pre-Metamorphic Tadpole Brainstem Preparations

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In terrestrial vertebrates, the development of functional respiratory neural networks must occur before pulmonary breathing is established. Research suggests that respiratory networks are particularly sensitive to hypoxic perturbations, but the impact of early hypoxic ‘experience’ on respiratory network development remains elusive due, in part, to the technical difficulties inherent in accessing embryonic neurons. Amphibians, which undergo pulmonary development during metamorphosis, offer a unique opportunity to study maturation of evolutionarily conserved neural networks supporting pulmonary ventilation. Using L. catesbeianus (Bullfrog), our lab has sought to gain insight into the effects of hypoxic stimulation on central respiratory network development. Pre-metamorphic tadpoles utilize gill and cutaneous breathing as the lungs are rudimentary, and yet evidence exists that lung motor activity is functional at early stages. Our cranial nerve recordings (CN’s VII & X) from in vitro brainstem preparations showed that lung bursting occurs at low frequency in pre-metamorphic tadpoles (TK stages 5-10), suggesting that the lung rhythm generator is functional but inhibited. Exposure of in vitro pre-metamorphic brainstems to acute hypoxia (10 min; 5% O₂ + 3% CO₂ + Bal N₂) doubled lung burst frequency (freq/min: base: 0.7±0.5; hypoxia: 1.5±1.1) with no effect on buccal (gill) rhythms. Remarkably, we discovered that following acute hypoxia, lung burst frequency persisted above baseline levels beyond 3 hours (freq/min: 3h post: 1.3±0.6), suggesting long-term changes in the underlying respiratory networks. Moreover, episodes of lung bursting, a marker of network maturity, occurred with higher frequency following acute hypoxia (freq/10min: base: 1.5±1.2; 3h post: 3.25±1.3). We hypothesize that cellular and synaptic changes occurring at the level of specific populations of motoneurons act to facilitate expression of adult-like lung motor patterns post-hypoxia.

This work was supported by an NSERC Discovery Grant awarded to R. Kinkead.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Abstract No. 8

Cysteine Oxidation in the Underlying Pathophysiological Mechanisms of Kidney Fibrosis Induced by Chronic Intermittent Hypoxia

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Background: Chronic intermittent hypoxia (CIH), hallmark of Obstructive Sleep Apnea (OSA), is a major contributor for OSA co-morbidities, namely hypertension and kidney disease (Dewan et al., 2015). Hypoxic cycles and subsequent reoxygenation lead to oxidative status, influencing cysteine (Cys) redox cycle. Upon oxidation, Cys may form disulfides with other thiols or react with sulfhydryl groups in proteins (protein S-cysteinylation - CysSSP). Protein S-cysteinylation is reversible, protecting plasma proteins from irreversible oxidation (Rossi et al., 2009). Plasma albumin of OSA patients show a decrease in sulfhydryl groups (Faure et al., 2008) but its relationship with kidney disease is unknown.

Aim: To evaluate the relationship between renal Cys_red/Cys_Ox ratio, plasma CysSSP and renal fibrosis in rats exposed to CIH.

Methods: 12 male Wistar rats (Rattus norvegicus), aged 8-12 weeks, were randomly assigned and divided into two groups: Group I - CIH conditions and Group II - normoxic (Nx) conditions (21% O2 and 79% N2). CIH animals were exposed to 10.5 h/day of CIH, for 21 days. At this time-point, rats already present established systemic hypertension (Diogo et al., 2015). Cys_red/Cys_Ox was quantified in plasma, medullar and cortical kidney samples by HPLC-FD (Dias et al., 2014). Collagen 1A1 and fibronectin gene expressions were analyzed by qPCR.

Results: CIH group presented a lower Cys_red/Cys_Ox in the renal cortex (p<0.01; Unpaired t-test) when compared with Nx group (0.65±0.05 vs 1.75±0.245, respectively). Collagen 1A1 and fibronectin (p<0.05) were overexpressed in the renal cortex of rats submitted to CIH. Additionally, an increase in plasmatic CysSSP (p<0.01) was observed in CIH (53.77±4.813 μM vs 36.36±1.333 μM; CIH vs Nx).

Conclusions: Cortical cysteine redox cycle is impaired in CIH-induced kidney fibrosis and might be monitored by plasma CysSSP. Together, these findings suggest the involvement of Cys oxidation in the underlying pathophysiological mechanisms related to hypertension and kidney disease in OSA.


Funding: NRC and CGD are supported by Fundação para a Ciência e a Tecnologia (FCT) - PD/BD/114257/2016 and PD/BD/105892/2014 fellowships. The work was funded by CEDOC and iNOVA4Health - UID/Multi/04462/2013, a program financially supported by FCT, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement (Ref: 201601-02-021).

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Molecular Characterization and Localization of Ectonucleotidases in the Rat Carotid Body: Regulation by Chronic Hypoxia

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The carotid body (CB) chemoreflex maintains blood PO2 and PCO2/H+ homeostasis and becomes sensitized during ventilatory acclimatization to hypoxia (VAH). Purinergic signaling via ATP and adenosine plays a pivotal role in shaping the afferent discharge at the sensory synapse containing chemoreceptor (type I) cells, glial-like type II cells, and sensory (petrosal) nerve endings. However, little is known about the ectonucleotidase enzyme family that controls synaptic nucleotide levels. In this study, we compared expression levels of ectonucleoside triphosphate diphosphohydrolases (NTPDases1,2,3,5,6) and ecto-5'-nucleotidase (E5’Nt/CD73) mRNA expression in juvenile rat CB versus brain, petrosal ganglia, sympathetic (superior cervical) ganglia, and a sympathoadrenal chromaffin (MAH) cell line using quantitative PCR (qPCR). The surface-located members NTPDase1,2 and E5’Nt/CD73 were among the highly expressed ectonucleotidases compared to low NTPDase3 expression in whole CB extracts. In CB sections and dissociated CB cultures, NTPDase2,3 and E5’Nt/CD73 immunoreactivity were localized to the periphery of type I clusters, and in association with sensory nerve fibers and/or isolated type II cells. Interestingly, in CBs obtained from rats exposed to chronic hypobaric hypoxia (~60 kPa, equivalent to 4,300m) for 5-7 days, there was a significant upregulation of NTPDase3 and E5’Nt/CD73 mRNA, but a downregulation of NTPDase1 and NTPDase2 relative to normoxic controls. We conclude that the rat CB predominantly expresses surface-located NTPDase1,2,3 and E5’Nt/CD73. Moreover, differential regulation of these enzymes during chronic hypoxia may contribute to CB plasticity during VAH via control of synaptic ATP, ADP and adenosine pools.

ACKNOWLEDGMENT
This work was supported by grants to C.A.N. from the Canadian Institutes of Health Research (MOP 142469) and to C.A.N. and G.B.M. from the Natural Sciences and Engineering Research Council of Canada.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Carotid Body Long-Term Facilitation Without CIH Preconditioning: Primarily a Post-Synaptic Mechanism Involving Phosphorylation of TRPV1 and Other Post-Synaptic Receptors, Independent of H₂O₂

A. Roy and R. J. A. Wilson

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BACKGROUND: Cardiorespiratory diseases, including sleep apnea, are associated with increased carotid body sensitivity and enhanced sympathetic activity but mechanisms responsible are unknown. We have demonstrated stimulus-dependent changes in carotid sinus nerve output in en bloc carotid body preparations, without chronic intermittent hypoxia (IHX) preconditioning, and report that acute IHx with concurrent hypercapnia (Hc) causes sustained increase in carotid sinus nerve activity (i.e. sensory LTF).

AIM: Determine the cellular mechanisms responsible for induction and maintenance of carotid body sensory LTF and compare to those described for sensory LTF requiring CIH preconditioning.

METHODS: All experiments were performed in carotid bodies from naïve rats using our novel artificially-perfused en bloc preparation which allows precise control of tissue PO₂ and PCO₂. Sensory LTF was induced with 10 bouts of IHx-Hc on a normocapnic background or IH on a hypercapnic background. Drugs were delivered before induction stimuli or once sensory LTF was established (maintenance phase).

RESULTS: The induction phase of sensory LTF was highly dependent on PKC activation; blocking Gq receptors (5HT2 and AT1) and ROS production (normally increasing PKC), or inhibiting ongoing PKC activity with GF109203X, prevents expression of LTF. Once established, sensory LTF was independent on ROS, 5HT2 and AT1 receptors, but reversed by GF109203X, and P2X2/3 and TRPV1 receptor blockers. While PKC and P2X2/3 are required for the carotid body to mount normal responses to acute Hx-Hc, TRPV1’s role is limited to sensory LTF. Importantly, neither induction or maintenance of sensory LTF is dependent on H2O2.

CONCLUSIONS: PKC activity, but not H₂O₂, is required to initiate and sustain sensory LTF. As TRPV1 receptors are located exclusively on chemosensory afferents, and sensory LTF depends on P2X2/3 receptor activation, we propose that sensory LTF in carotid bodies in naïve rats is largely a post-synaptic mechanism involving phosphorylation of TRPV1 and other post-synaptic receptors.

R.J.A.W. is an Alberta Innovates Senior Scholar. Funded for this project is provided by CIHR.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Contribution of Relative Leptin and Adiponectin Deficiencies to Chronic Intermittent Hypoxia in Premature Infants

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Instability of the respiratory network from low central respiratory drive and the greater contribution of the carotid body on baseline breathing leads to respiratory instability in premature infants presenting as apnea and periodic breathing. The smallest and the youngest premature infants have increased frequency of apnea and periodic breathing and associated oxygen desaturations for many weeks after birth. Leptin is a central respiratory stimulant and increases CO₂ sensitivity in adults, and adiponectin protects the lung from vascular leak, oxidative injury and vascular remodeling. Low respiratory central respiratory drive, and lung injury creates the perfect storm for chronic intermittent hypoxia to occur. The smallest and the youngest infants have minimal adipose tissue and thus have very low leptin and adiponectin levels. We sought to determine the role of leptin on CO₂ and adiponectin on lung protection during early development. On postnatal day (PND) 15, newborn rat pups were exposed to exogenous leptin 7ug/kg/day (3 days, mini-osmotic pump), and on PND 18, ventilatory responses to hypercapnia, In separate group animals, regional expression of signal transducer and activator of transcription 3 (pSTAT3, biomarker for leptin receptor activation) in the locus coeruleus (LC) and nucleus tractus solitaries (nTS) after 60 mins exposure to hypercapnia (5%CO₂/21% O₂) was determined. Exogenous leptin 1) increased plasma leptin levels 4-fold, 2) increased hypercapnic minute ventilation. Leptin exposure did not significantly affect body weight. Hypercapnia (7%CO₂/ 21% O₂) exposure for 60 mins increased protein expression for pSTAT3 in the nTS and LC. We determined the effect of exposure to bleomycin (model of chronic lung disease), or hypoxia (13%O₂; model of pulmonary hypertension) from birth on adiponectin receptor expression in the lung of p14 and p21 animal, s respectively exposure to bleomycin expression of adiponectin receptor 2 (AdipR2) in the lung of new rats after exposure. Both exposures significantly decreased AdipR2 expression when compared to saline treated controls. These preliminary data suggest that 1) leptin modifies ventilation, and hypercapnia activates leptin receptors in CO₂ sensitive areas in the brainstem in 2-3 old rats, 2) in 2 models of lung injury from birth, AdipR2 expression is downregulated. Experiments are being done to further explore the development profile of these responses/ References:


Abstract No. 12

A New Role of Chronic Intermittent Hypoxia on Pathology: Spontaneous Tumorigenesis and Consequences

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Physiological systemic effects of carotid body (CB) activation have long been studied. CB implication in respiratory diseases that involve hypoxic situations has turned into a field of high interest. Chronic sustained hypoxia in chronic obstructive pulmonary disease and chronic intermittent hypoxia (CIH) in obstructive sleep apnea (OSA) are some examples. OSA is a high prevalent breathing disorder linked with cardiovascular, metabolic, and neurocognitive diseases. Recently, it has been proposed a relationship between CIH and cancer. Limited studies have evidenced that CIH augments growth and metastasis rate of implanted tumors in mice (Almendros et al., 2017). In OSA patients, although with some discrepancies, association between OSA and cancer incidence/mortality has been reported (Nieto et al., 2012). These apparent disparities could be due to the large number of comorbidities present in OSA patients, their uncontrolled variables and the type of cancers. Trying to simplify the complex pathological human situation, CIH as a single variable has been used to evaluate its effects on spontaneous tumorigenesis and plasma biomarkers. In an old outbreed murine model, two intensities of CIH were applied (12%O2, mild and 7.5%O2, severe) mimicking two stages of OSA patients pathological situation. Long term (3 months) severe CIH spontaneous tumor (lung, liver, skin) incidence was 62.3±13.0%, vs controls 36.4±12.7% (p=0.007). Among all, only lung tumors incidence was statistically significant 28, 3%±12.1 vs controls 12.7%±8.8 (p=0.04). Plasma levels of C reactive protein (CRP) and tumor markers, carcinoembryonic antigen (CEA) and neuron specific enolase (NSE), increased in mice with tumors; but their increment in CIH mice was attenuated in a hypoxic intensity related manner. These findings could alert about the necessity of evaluating cancer incidence and type of cancer in OSA patients; and that the minor increase of plasma tumor markers with CIH intensity could under evaluate cancer staging.

Nieto et al, (2012). Am J Respir Crit Care Med 186:190-4

Supported by grants: BFU2015-70616R (MINECO-FEDER); CIBER CB06/06/0050 (ISCiii); Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Lysophosphatidic Acid Stimulates the Carotid Body: Linking Inflammation and Carotid Sinus Nerve Activity


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Background: As systemic levels of inflammatory mediator lysophosphatidic acid (LPA) increases with inflammatory lung disease (1), LPA is a TRPV1 agonist (2) and TRPV1 is expressed in CB (3), we hypothesized that LPA stimulates CB. Methods: We tested our hypothesis by evaluating the effects of LPA on carotid sinus nerve and phrenic nerve discharge in novel en bloc carotid body perfused and in situ decerebrate, vagotomized, rodent dual-perfused preparations, respectively. In carotid sinus nerve intact and denervated in situ preparations, carotid bodies and the brainstem were perfused separately. The carotid bodies were perfused with normoxia/normocapnia and the brainstem was perfused with hypocapnia to induce apnea and thus increase phrenic burst frequency sensitivity to carotid body activation. LPA (5µM) was injected into the carotid body perfusate. To determine whether non-TRPV1 LPA receptors might also be involved, we tested the effects of LPA in the presence of the TRPV1 antagonist AMG9810 using the en bloc preparation. We also performed RT-PCR for LPA receptors (LPAr) 1-4 in rat carotid bodies, petrosal ganglia and superior cervical ganglia. Results: LPA delivered directly to the carotid body circulation in in situ preparations significantly (p<0.05) increased phrenic nerve burst rate (from 1.2±0.8 burst min\(^{-1}\) to 20.8±2.9 burst min\(^{-1}\)) and amplitude (from 1±0.5 to 2.5±0.5 normalized units). In carotid body denervated preparations, LPA had no effect. Blocking TRPV1 receptors reduced but did not eliminate the effects of LPA on carotid sinus nerve activity in the en bloc preparation. RT-PCR revealed expression of LPAr 1, 3, and 4 in the carotid body, LPAr 3 in the petrosal ganglia and LPAr 1, and 3 in the superior cervical ganglia. Conclusion: Our data suggest that the carotid bodies are sensitive to LPA via TRPV1 and LPAr receptors which may involve CB in the neural components of inflammatory pulmonary diseases.

References:

R.J.A.W. funding is provided by Canadian Institute for Health Research. R.J.A.W. is an Alberta Innovates Senior Scholar. N.G.J. postdoctoral salary was supported by Alberta Innovates, Canadian Allergy Asthma and Immunology Foundation, T Chen Fong Foundation, Campus Alberta Neuroscience, Alberta Children’s Hospital Research Institute and the Canadian Thoracic Society.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Abstract No. 14

**Carotid Sinus Nerve Resection Decreases Weight Gain and Fat Mass in a Rodent Model of Obesity**

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Obesity contributes to significant morbidity and mortality worldwide. The therapeutic options to treat this pandemic are scarce. Our group has demonstrated that abolishment of carotid body (CB) activity, through carotid sinus nerve (CSN) resection, prevents and reverses insulin resistance and glucose intolerance in animal models of metabolic diseases (Ribeiro et al., 2013; Sacramento et al., 2017). Herein, we investigated the effects of CSN resection on weight gain and on the pathophysiological mechanisms that can contribute to the metabolic beneficial effects, in an animal model of obesity. For this, Wistar rats were subjected to 10 weeks of high-fat (HF, 60%-lipid rich diet) (5.1Kcal/g) or standard diets (2.85Kcal/g) and afterwards submitted to bilateral CSN resection. CSN resection was confirmed by the absence of ventilatory responses to hypoxia. After surgery, animals were kept under the respective diets and insulin sensitivity, glucose homeostasis, caloric intake and body weight were monitored. At terminal experiments, animals were anaesthetised and adipose tissue depots were collected, weighted and stored for further analysis. As expected, HF diet produced a significant increase in weight gain. CSN resection decreased weight gain in the HF and control animals (40% and 13%, respectively), an effect that is not due to decreased caloric intake. Moreover, CSN resection restored insulin sensitivity and glucose homeostasis and decreased the total fat amount of the HF animals (35%), due to a reduction in perienteric (56%), perinephric (35%) and subcutaneous (35%) adipose tissue mass. Additionally, CSN denervation induced a reduction in lipid deposition in the liver by 32%. We can conclude that abolishment of CB activity positively impacts on weight gain due to a significant decrease in adipose tissue mass. Also we suggest that the decrease in lipid deposition in the liver can contribute to the improvement of metabolic features in CSN-denervated animals.


J.F. Sacramento and B.F. Melo were supported by the Portuguese Foundation for Science and Technology grants PD/BD/105890/2014 and PD/BD/128336/2017 respectively.

* Both authors have contributed equally to this work.
Overactivation of Carotid Sinus Nerve Activity in High-Fat Animals is Mediated by A2 Adenosine Receptors

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Our group have described that carotid sinus nerve (CSN) resection prevents and reverses insulin resistance in prediabetes animal models (Ribeiro et al., 2013; Sacramento et al., 2017). Herein we have investigated if the high-fat diet modifies the CSN chemosensory activity in basal conditions and in response to hypoxia and hypercapnia and, if this effect is mediated by A2 adenosine receptors.

Two groups of male Wistar rats were used. The control group fed a chow diet and the high-fat (HF) group fed a 60% lipid-rich diet (21 days). After this period, insulin sensitivity was evaluated by an insulin tolerance test. To perform the CSN activity recordings, CB-CSN was dissected and transferred to a recording chamber superfused (37°C) with Tyrode bicarbonate equilibrated with normoxia (20%O2+5%CO2+75%N2). Chemoreceptor activity was identified (spontaneous generation of action potentials at regular intervals) and confirmed by its response to hypoxia (0%O2). The effects of hypoxia (0 and 5%O2) and hypercapnia (10%CO2) were investigated in control and HF animals as well as the effects of ZM241385 (300nM; an A2 antagonist) on the CSN activity in normoxia and hypoxia (0%O2)-evoked CSN action potentials. All animals were killed by an intracardiac overdose of pentobarbital sodium (60 mg/kg i.p.).

HF diet increased basal CSN activity by 137.50%. The chemosensory response to intense hypoxia (0% O2) was not modified by HF diet. In contrast, the CSN chemosensory response to moderate hypoxia (5% O2) was higher in the HF group. The latency time and the time to peak were significantly decreased in the HF group in response to intense and moderate hypoxia. The CSN responses to hypercapnia was not modified by HF diet. ZM241385 decreased basal CSN activity in the HF group. ZM241385 also decreased the CSN chemosensory response to hypoxia (0% O2) in control and HF group.

The HF diet increases the basal CSN activity and the CSN response to moderate hypoxia, as well as the responsiveness to hypoxia, an effect that is mediated by A2 adenosine receptors.


JF Sacramento by PD/BD/105890/2014.
Therapeutic Targeting of Carotid Body for Treating Sleep Apnea in a Pre-Clinical Model

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Sleep apnea, which is the periodic cessation of breathing during sleep, is a major health problem affecting over ten million people in the USA and is associated with several sequelae including hypertension and stroke. Clinical studies suggest that abnormal carotid body (CB) activity may be a driver of sleep apnea. As gaseous molecules are important determinants of CB activity, aberrations in their signaling could lead to sleep apnea. We tested this possibility in mice deficient in heme oxygenase-2 (HO-2), which generates the gaseous molecule carbon monoxide (CO). A majority (75%) of the HO-2 /- mice displayed frequent apnea of equal or greater than 20 events per hour, whereas only 5% of the WT mice exhibited such common apnea. Likewise, 56% of the HO-2 /- mice had a hypopnea (breathing event with ≥ 30% reduction in tidal volume) index greater than 80. In contrast, only 2.5% of the WT mice displayed a similar hypopnea index. Incidence of apneas and hyponeas was high during sleep as identified by electroencephalography. HO-2 /- mice had both obstructive and central sleep apnea, and the incidence of former was greater than the latter. Inhibition of CB activity either by genetically ablating the H2S-synthesizing enzyme cystathionine-γ-lyase (CSE) or pharmacologic blockade of CSE reduced CB sensory activity, and normalized breathing in HO-2 /- mice. Pharmacokinetic properties of CSE inhibitors showed 100% bioavailability. These observations demonstrate that pharmacological targeting CB prevents to apneas. (Supported by NIH-UH3-HL90554 and PO1-HL90554).
Contrasting Effects of Acute and Chronic Progesterone Administration on Apnea Frequency in Newborn Male and Female Rats

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We previously showed that acute administration of progesterone decreases the frequency of apnea in 1-day old rats but increases apnea frequency in 12 day-old male rats (Bairam 2013). However, chronic exposure to progesterone through the milk of lactating dams reduced apnea frequency in 10 day-old male rats (Lefter 2007). We tested the hypothesis that in 12 days old rats (n=77), the effects of acute and chronic progesterone on apnea frequency are sex-specific. In addition, because progesterone was suggested as a potential avenue for apnea treatment in newborn babies when caffeine is ineffective, we also tested the effect of an acute injection of caffeine in pups that received a chronic administration of progesterone. We used whole-body plethysmography to measure ventilation, apnea frequency (spontaneous and post-sigh apneas) and duration (sec) following acute (4mg/kg, i.p) or chronic (4mg/kg, orally once/day from day 3 to 12) progesterone administration. The effects of caffeine (10mg/kg, i.p.) were assessed in 12 day-old male and female rats after chronic progesterone administration. Basal ventilation was not affected by acute or chronic administration of progesterone. The frequency of spontaneous apnea was increased by acute administration of progesterone in male (p=0.006) and female (p=0.016) rats, and decreased by chronic progesterone administration in male (p=0.02) but not in female rats. Interestingly, acute caffeine administration decreased apnea frequency in female rats after chronic progesterone administration (p=0.04), but not in males. We conclude that i. The effects of progesterone on apnea frequency in newborn rats depend on the duration of treatment, ii. Chronic administration of progesterone decreases apnea frequency in newborn male rats, but not in females, and iii. Caffeine reduces apnea frequency in female rats after chronic administration of progesterone. These observations may be related to the interplay of nuclear and membrane progesterone receptors and their potential sex-specific effects in newborn mammals. (CIHR-MOP:119272)

Abstract No. 18

Leptin Increases Blood Pressure Acting in the Carotid Body

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**Rationale:** Obesity leads to cardiovascular morbidity and mortality acting via multiple mechanisms including hypertension and obstructive sleep apnea. Obesity leads to high levels of adipokine leptin. Increased leptin levels have been previously reported in sleep apnea and implicated in increased sympathetic activity and the pathogenesis of hypertension. However, mechanisms of the effects of leptin on blood pressure are unclear. The carotid bodies (CB) express leptin receptor (LepR), but the mechanisms and consequences of leptin action in CB are unknown. We have previously demonstrated that leptin enhances carotid sinus nerve activity in response to hypoxia and this effect is abolished by non-selective blockers of transient receptor potential (TRP) channels (Shirahata et al., 2015). We hypothesized that high levels of circulating leptin signal via LepR in CB leading to hypertension. **Methods:** 1) Male C57BL/6J mice (n=6) were implanted with telemetry in the left femoral artery for blood pressure monitoring at baseline, during leptin infusion (120ug/day for 3 days via a SC pump) before and after CB denervation; 2) LepR deficient db/db mice were implanted with telemetry; blood pressure measurements were performed followed by infection with adenovirus carrying the LepR gene (Ad-LepR, n=5) or luciferase (control, Ad-Luc, n=5) followed by BP measurements. **Results:** 1) In mice with intact CB, leptin increased mean arterial pressure by 13 mm Hg during the day and by 16 mm Hg at night (p = 0.003 for the effect of leptin). CB denervation completely abolished leptin-induced hypertension (p < 0.001). 2) LepR expression in CB of db/db mice significantly increased blood pressure. **Conclusions:** Leptin increases blood pressure acting via LepR in the CB. Experiments to examine the role of TRP channels in leptin-induced hypertension are currently in progress.
Divergent Effects of Myo-Inositol on Respiratory Neural Control During Postnatal Development

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Myo-inositol is a nutritional agent that can also be formed from the breakdown of glucose – it ultimately forms structural lipids and intracellular second messengers and plays an important role in neural development and function. Prior studies have demonstrated beneficial effects of myo-inositol supplementation on lung development in preterm infants, however, its effects on respiratory control are unknown. We tested the hypothesis that myo-inositol administration during the postnatal period would affect the acute hypoxic (HVR) and hypercapnic (HCVR) ventilatory responses in neonatal mice. From the day after birth, mice received subcutaneous injections myo-inositol [100 mg/kg/day] or vehicle for the first two postnatal weeks. The acute [5 min, 10% O₂] HVR and HCVR [5 min, 5% CO₂] were assessed at one and two weeks of age. In one week old mice, myo-inositol abolished the acute HVR compared to vehicle treated pups. In contrast, myo-inositol had the opposite effect at two weeks of age when the acute HVR was enhanced compared to age-matched vehicle treated mice. These effects were unique to male mice, whereas females appeared unaffected by myo-inositol. Further, myo-inositol did not affect the HCVR in either age group suggesting a selective effect on hypoxic sensitive pathways. These data demonstrate a divergent effect of myo-inositol treatment on respiratory control during postnatal development, characterized as an inhibitory effect on the HVR at one week of age, followed by a transition toward an augmenting effect by two weeks. Such contrasting effects of myo-inositol reveal potentially deleterious vs beneficial effects on respiratory neural control at specific stages of postnatal development. These data may have implications for the way that postnatal changes in dietary myo-inositol could influence the severity of respiratory morbidities associated with abnormal respiratory control that are common to preterm infants such as respiratory distress and apnea of prematurity.

Acknowledgements to funding bodies: The Gerber Foundation.

*Where applicable, the authors confirm that the experiments described here have received ethical approval.*
Protection from SIDS: Perhaps it is in the Milk

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Premature infants have a 2-3 fold increased incidence of SIDS compared to infants born at term. Moreover, low birthweight male infants (<2500 grams) have the greatest risk of dying of SIDS. Prone sleep position, maternal smoking, not breast feeding, bed sharing, are considered the 4 major modifiable risks for dying of SIDS. Reduction in each and all of these risk factors reduces the risk of SIDS. While studies have been done to better understand the mechanism attributing to the increased risk for prone sleep position, maternal smoking and bed sharing, the protective effect of breastfeeding is not completely understood. Breastfeeding substantially reduces the odds of dying from SIDS with a summary odds ratio of 0.27 (95% CI: 0.24–0.31) for infants who exclusively received breastmilk for any duration. Infants who breastfeed arouse more easily from active sleep than formula fed infants at 2-3 months of age, and breastfeeding also confers protection from infection with immunoglobins and cytokines, and improves innate immunity. Serotonin neurons in the medullary raphe are sensitive to CO₂ and the sensitivity of these neurons in slices and culture, parallels the increase in the hypercapnic ventilatory response in newborn rats. Focal inhibition of 5-HT neurons and genetic studies to reduce or eliminate 5-HT expressing neurons in the brainstem results in marked decreased in CO₂ sensitivity. Local injections of leptin in the solitary tract nucleus increased CO₂ sensitivity. Moreover, the reduction in the number of these neurons have been found in infants who have died from SIDS. Breastmilk contains leptin and leptin plasma levels increase in newborns after a breastmilk feed. In contrast, formula does not contain leptin and infants who are forumula fed have significantly lower plasma leptin levels than those who are breastfed. SIDS occurs during sleep and most often after feeding. Leptin crosses the blood brain barrier. A rise in environmental CO₂ and resulting respiratory depression is thought to be operative in SIDS. Higher leptin levels after a breastfeed could theoretically increase central CO₂ sensitivity of brainstem neurons. In this short communication, I will present data to support the rationale that in addition to the other protective factors in breastmilk, leptin in breastmilk may also be important in protecting the infant from SIDS during sleep.

References:


POSTER PRESENTATIONS
Hypoxia-Induced Rise in $[Ca^{2+}]$ in Rat Carotid Body Glomus Cells: Does it Involve Inhibition or Activation of BK/Kv?

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In rat glomus cells, hypoxia inhibits TASK, a background K$^+$ channel, to depolarize the cells and initiate and sustain the hypoxic response (i.e., rise in $[Ca^{2+}]$). In addition, it is thought that BK/Kv can be basally active and that hypoxia inhibits them to cause depolarization. The observed peak hypoxic response has been described to be determined by either (1) the removal of the hyperpolarizing force because hypoxia inhibits BK/Kv, or (2) the strong hyperpolarizing force because depolarization activates BK/Kv. To distinguish between these two opposite schemes, we studied the effect of K$^+$ channel inhibitors on hypoxia-induced rise in $[Ca^{2+}]$. Anoxia and various levels of hypoxia elevated $[Ca^{2+}]$ as expected. In the presence of TEA/4-AP, the rise in $[Ca^{2+}]$ by hypoxia was strongly potentiated (≈3-fold), indicating that activation of BK/Kv limited the rise in $[Ca^{2+}]$. In the presence of guangxitoxin-1E (GxTX), the rise in $[Ca^{2+}]$ was also markedly enhanced (≈2-fold), indicating that activation of Kv2 alone can limit the hypoxic response. This is consistent with the finding that Kv2 is a major Kv subtype in rat glomus cells. To show that hypoxia activates BK/Kv, we studied the effect of anoxia on BK/Kv in inside-out and cell-attached patches. Contrary to earlier reports, anoxia had no effect on BK in inside-out patches. When BK was allowed to become basally active in cell-attached patches by depolarizing the membrane with high extracellular [KCl], anoxia caused reversible activation, showing that hypoxia-induced depolarization activated BK. We were unable to test the effect of anoxia on Kv, because of very low single channel conductance levels under our experimental conditions. These results do not support the mechanism in which hypoxia inhibits BK/Kv to sustain the hypoxic response, but support the mechanism in which hypoxia activates BK/Kv to limit the hypoxic response in rat glomus cells.

(Funded by NIH)

*We confirm that the experiments described here have received ethical approval.*

*(If the first author is able to attend the meeting, he (Jiaju Wang) will present it)*
Expression and Function of TASK-1 and TASK-3 in Rabbit Carotid Body Glomus Cells


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Glomus cells isolated from rabbit and rat/mouse carotid bodies have been used to study the role of ion channels in hypoxia sensing. Studies have shown that hypoxia inhibits the inactivating K⁺ channels (Kv3.4/4.1/4.3) in rabbits, but inhibits TASK (and possibly BK/Kv) in rats to elicit the hypoxic response. Thus, TASK may be absent in rabbit glomus cells, which is consistent with no reported TASK activity. To confirm this, we isolated glomus cells from young rabbits and studied the expression of TASK-3 mRNA in the whole CB. We then recorded changes in intracellular [Ca²⁺] and TASK (cell-attached patches) in normoxia and hypoxia. RT-PCR showed that rabbit CB expressed mRNA for TASK-3. We also detected the expression of Kv2.1, Kv3.1 and Kv3.3, indicating that many different Kv subtypes are expressed. In rabbit glomus cells in which 20 mM KClo elevated [Ca²⁺], anoxia also elicited a strong rise in [Ca²⁺]. In cell-attached patches with 140 mM KCl in the pipette and 5 mM KCl in the bath solution, opening of several types of ion channel was observed. The single channel conductance levels were 16-pS, 34-pS, and 42-pS TREK-like channels were also observed. In inside-out patches with high [Ca²⁺], BK openings were activated. The 42-pS channel opened spontaneously and briefly under our recording condition and is most likely Kv. The 16-pS and 34-pS channels showed properties similar to those of TASK-1 and TASK-3, respectively. TASK activity was very low (Po<0.02), compared to that in rat glomus cells (Po: 0.3-1.5). Anoxia reduced TASK activity by ~60%. Overall, these results show that TASK-1 and TASK-3 are expressed in rabbit glomus cells, but their activity at rest is very low. We conclude that, although inhibited by hypoxia, TASK-1 and TASK-3 contribute very little to the hypoxic response in rabbit glomus cells.

(Funded by NIH and Chicago Medical School)

We confirm that the experiments described here have received ethical approval.
Molecular and Functional Analysis of T-Type Ca\(^{2+}\) Channels in Rat Carotid Body


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Extracellular calcium influx is a necessary step in carotid body (CB) type I cells during hypoxic activation and neurotransmitter release, triggering the chemosensory reflex which maintains cardiorespiratory functions during low pO\(_2\). Although both, high (HVA: L, N, P/Q and R type) and low voltage-activated (LVA) Ca\(^{2+}\)-channels are present in CB type I cells, no much it is known about the relevance of the LVA T-type channels in this organ. Three different genes codify for T-type channels (Cav3.1, Cav3.2 and Cav3.3), but only Cav3.2 channels have been described in detail in CB. It has also been reported that chronic hypoxia (CH) up-regulates Cav3.2 expression in PC12, chromaffin and pulmonary artery smooth muscle cells and, more recently, in CB.

In the present study, we have analyzed the expression and localization of T-type Ca\(^{2+}\)-channels in the rat CB and assessed their functional significance in response to acute and chronic hypoxia. By immunocytochemistry and RT-PCR techniques we provide evidence for a predominant presence of Cav3.1 in type I cells, lower Cav3.2 expression, and absence of Cav3.3. Rats’ exposure to CH up-regulates Cav1.2 channels (L-type Ca\(^{2+}\)-channels), diminishes Cav3.1 and does not change Cav3.2 expression. Hypoxia activated CB response can be 50% reduced by specific T-type channel inhibitors: low concentration of Ni\(^{2+}\), mibefradil and TTA-A2. We demonstrated augmented responses in CH treated rat CB, which also are 50% sensitive to the same inhibitors. In cultured CB type I cells, patch-clamp recordings showed the presence of T-type currents reversibly blocked by mibefradil. However, LVA currents were only detected in \(\approx\)10% of the studied cells.

We conclude that in rat CB, T-type calcium channel contributes to basal and CH augmented type I cell excitability and secretory response. Cav3.1 could be the predominant T-type Ca\(^{2+}\)-channel present in rat CB.

Acknowledgements: MINECO-FEDER BFU2015-70616R; ISCiii CIBER CB06/06/0050; SAF2016-77233-R and Programa PROMETEO II (project 2014/014 Generalitat Valenciana).

Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Mitochondrial Complex I Dysfunction and Oxygen Sensing in a FASTK-Deficient Mice Model

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The molecular mechanisms underlying O2-sensing by carotid body (CB) chemoreceptors remain undetermined. Mitochondria have been related due to the sensitivity of CB to electron transport chain (ETC) blockers. Furthermore, ETC is one of the major sources of Reactive Oxygen Species, proposed as mediators in oxygen-sensing. Fas-activated serine/threonine phosphoprotein is a sensor of mitochondrial stress that modulates protein translation to promote survival of cells exposed to adverse conditions. A translational variant of Fas-activated serine/threonine kinase (FASTK) is required for the biogenesis of ND6 mRNA, the mitochondrialencoded subunit 6 of the NADH dehydrogenase complex (Complex I). Ablating FASTK expression reduced Complex I activity in vivo by >50% (Simarro et al., 2010). Here, we have tested the hypothesis of Complex I participation in O2-sensing structures by studying the effect of acute hypoxia in FASTK-/- knockout mice.

In vivo plethysmography data showed that under normoxic conditions, knockout mice had a lower minute volume than wild mice (MV; 1257±95 vs 1774±198 ml/min/kg; n=6; p<0.05) without CB catecholaminergic activity differences. However, MV induced by acute hypoxia (12%O2) was similar (3607±443 vs 3916±308 ml/min/Kg). Conversely, they exhibited a significantly lower ventilatory response to hypercapnia (5% CO2; MV=2798±190 vs 3984±579; p<0.05). Oxygen consumption (VO2) in normoxia, hypoxia or hypercapnia was comparable but CO2 production (VCO2) was also significantly lower in knockout mice (42.72±6.59 vs 70.41±6.79; p<0.05). In vivo Hypoxic Pulmonary Vasoconstriction was preserved in knockout animals. Pulmonary artery contractility in vitro, using small vessel myography, showed similar responses to 80mM KPSS (2.79±0.43 vs 2.63±0.26 mN; n=6). Considering the effect of rotenone on preexisting PGF2 contraction as an indicative of ETC functionality, we found a significantly decreased relaxation in knockout mice (42.7±5.5 vs 73.4±8.6, as % of PGF2 response, n=6; p<0.05). In conclusion, FASTK-/- knockout mice maintain oxygen-sensing under hypoxic stress but partially lose CO2 response under hypercapnic stress.


Funded by BFU2015-70616R (MINECO/FEDER), CB06/06/0050 (ISCiii) and BIO/VA20/15; BIO/VA21/15 (JCyL).

Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Topical Application of Connexin-Based Hemichannel Blocker Reduces Carotid Body-Mediated Chemoreflex Drive in Rats

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The carotid body (CB) is the main arterial chemoreceptor involved in oxygen sensing. Upon hypoxic stimulation, the CB chemoreceptor cells release neurotransmitters to increase the action potential discharge frequency in sensory nerve fibers from the carotid sinus nerve. Identification of the precise molecular entity in charge of oxygen sensing is still a matter of debate. However, several ion channels have been shown to at least regulate the oxygen sensitivity in the CB. Connexins-based ion channels are constitutively express in the CB. However, there is a lack of causal evidence showing a regulatory effect, if any, of connexin-based ion channels in CB oxygen sensitivity. Therefore, we aimed to determine whether connexin-based ion channel blockers alter CB-mediated chemoreflex drive. To test this we studied the effects of acute CB topical application of a connexin hemichannel blocking agent (CHBa) on the CB-mediated ventilatory reflex response to several oxygen levels (F\textsubscript{O\textsubscript{2}} 21, 15, 12, 10 and 5 %) in adult male Sprague-Dawley rats (~200 g). In normoxic conditions, no effects of CHBa on respiratory tidal volume nor in respiratory rate was found. However, CHBa significantly impaired the CB-mediated chemoreflex response to hypoxia. Indeed, CHBa reduced both the hypoxic ventilatory response (HVR) gain and the maximum HVR by ~25% and ~50%, respectively. Our results showed that connexin-based hemichannels within the CB contribute to the CB-mediated chemoreflex response to hypoxia in rats. Also, our results support CB connexin-based ion channels as potential molecular entities to target in several CB-mediated diseases.

Funding: FONDECYT #1140275.

All the experiments were approved by the IACUC from the Universidad Autonoma de Chile.
Guinea Pig as a Negative Model to Study the Carotid Body Mediated Chronic Intermittent Hypoxia Effects


Department of Biochemistry and Molecular Biology and Physiology, University of Valladolid, Institute of Molecular Biology and Genetics-CSIC, CIBERES, ISCiii, Spain.

Chronic Intermittent Hypoxia (CIH) is one of the causes of the systemic arterial hypertension observed in the obstructive sleep apnea syndrome, which seems to be due to carotid body (CB) sensitization. Previous data show a hypofunctional guinea pig CB, lacking any response to acute hypoxia (Gonzalez-Obeso et al. 2017). Guinea-pig could be a negative model to study the CB mediated CIH effects. In this study, male Hartley guinea pigs were exposed to CIH (21%O₂-80s / 5%O₂-40s 8h/day; 30 days).

No changes in respiratory minute volume when acute hypoxia, hypercapnia or Dejours tests were observed by plethysmography after CIH exposure. No differences were found in mean arterial blood pressure or in tissue catecholamine (CA) content (CB, adrenal medulla, renal artery by HPLC). In vitro myography measurements of pulmonary, aortic or carotid arteries showed no changes on contraction (30μM phenylephrine) or relaxation (10μM carbachol) although plasma nitrites levels (39 vs 47μM; p<0.05) increased after CIH. Morphological differences observed in the carotid artery were mainly due to thickness in muscle layer.

However, an increased arterial pulse pressure (20.1 mmHg in air and 28.2 mmHg in 10% O₂; p<0.01), rise of heart rate (25%; p<0.001), norepinephrine plasma levels (18 vs 80 pmol/ml) and renal artery CA synthesis (32%; p<0.05) after CIH treatment were observed. These data would indicate a hypoxic activation of the sympathetic system nondependent of CB chemoreceptors.

These data, obtained by the first time in CIH guinea pigs, would suggest that they possess an oxygen sensing mechanism responsible for the sympathetic cardio-circulatory reflex, probably through stimulation of the central chemoreceptors. In conclusion, guinea pigs are an appropriate model to study the CB-dependent and nondependent effects induced by CIH.


Supported by grants: MINECO BFU2015-70616R; ISCiii CIBER CB06/06/0050

Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Abstract No. P7

**Lactate Sensing by Carotid Body Glomus Cells**

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The carotid body (CB) is a multimodal chemoreceptor organ in which glomus cells sense changes in blood O2, hypoglycemia, CO2, and pH. Recently, it has been suggested that CB glomus cells can also detect an increase in blood lactate, although the underlying mechanisms are unknown. We have investigated the effects of lactate on dispersed single glomus cells and in CB slices. Extracellular L-lactate (2-10 mM, sodium salt) increased glomus cell secretion rate, evaluated by amperometry, without occluding the secretory response to hypoxia and both stimuli (hypoxia and lactate) had additive effects. In accord with the amperometric data, isolated glomus cells responded to both, lactate and hypoxia, with an external Ca2+-dependent increase in cytosolic [Ca2+], as well as accumulation of NADH and reactive oxygen species (ROS). However, the kinetics of NADH increase induced by lactate were faster than those elicited by hypoxia. These data suggest that hypoxia and lactate increase NADH levels and activate glomus cells through separate signal transduction pathways. Increase in extracellular lactate seems to produce accumulation of lactate in the cytosol, which gives rise to NADH generated during the conversion of lactate to pyruvate. In contrast, hypoxia accumulates NADH primarily in the mitochondria, due to slow down of NADH/quinone oxidoreductase activity, which indirectly changes NAD(P)H levels in the cytosol, as shown previously (Fernández-Agüera et al., 2015). Activation of glomus cells by lactate may play an important role in CB-mediated regulation of respiration during exercise and in pathological conditions presenting lactic acidosis.


The research was supported by grants from the Botín Foundation, Spanish Ministry of Science and Innovation, and the European Research Council.

*The authors confirm that the experiments described here have received ethical approval.*
Abstract No. P8

Acute Effects of Systemic Erythropoietin Injections on Carotid Body Chemosensory Activity Following hypoxic and Hypercapnic Stimulation


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Peripheral carotid body (CB) chemoreceptors can detect changes in arterial blood gases. Upon stimulation CB chemoreceptors cells release one or more transmitters to excite sensory nerve fibers of the carotid sinus nerve. While several neurotransmitters have been described to contribute to the CB chemosensory process less is known about modulatory molecules. Recent data suggest that erythropoietin (Epo) is involved in the control of ventilation. Indeed, it has been shown that Epo could alter the cellular metabolism of neurotransmitters (i.e. catecholamines) in the CB. Furthermore, it has been shown that Epo can regulate the ventilatory reflex response to hypoxia/hypercapnia in humans and mice in a sex-dependent manner. In the present study, we aimed to determine whether exogenous applications of Epo modulate the hypoxic and hypercapnic CB chemosensory responses. Carotid sinus nerve activity was recorded in-situ from anesthetized adult male and female Sprague-Dawley rats (~350 g, n=8) before and after systemic administration of Epo (2000 IU/kg). CB-chemosensory sensitivity to hypoxia and hypercapnia was calculated by allowing the rat to spontaneously breathe FiO2 5-15% and FiCO2 3-7% gas mixtures, respectively. During baseline recordings at normoxia, we found no effects of Epo on CB activity both in male and female rats. In addition, Epo shown no effects in maximal CB response to hypoxia neither in CB oxygen sensitivity in male or female rats. On the contrary, Epo injections enhanced the maximum CB chemosensory response to hypercapnia only in female rats (before vs. after Epo, 72.5±7.1 Hz vs. 108.3±6.9 Hz, p<0.05). Contrarily in males, Epo has no effect on maximum CB chemosensory response to hypercapnia but significantly increases the response recovery times (time required to get to baseline activity following hypercapnic stimulation) from 2.1±0.1 s to 8.2±2.3 s (p<0.05). Together, our results suggest that Epo is a plausible modulator of the CB chemosensory response to hypercapnia.

Funding: FONDECYT #1150040, #1140275 and PROGRAMA DE COOPERACIÓN CIENTÍFICA ECOS-CONICYT C16S03.

All the experiments were approved by the IACUC from the P. Universidad Catolica de Chile.
Leptin in the Commissural Nucleus Tractus Solitarii Increases the Glucose Responses to Carotid Chemoreceptors Activation by Cyanide

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Contribution statement. §Equal contribution as the first author. *Corresponding author

The carotid glomus cells detect the PO₂ within the carotid sinus arterial blood and transmit this information to the CNS, activating multiple homeostatic mechanisms, including glucose regulation. It is well established that the protein hormone leptin is an adipose obese gene product that plays a key role in the regulation of energy balance and glucose homeostasis. Leptin and its receptor Ob-Rb have been found within the carotid glomus cells, but its role in glucose regulation and metabolism is still not clear. This study was done to determine whether the exogenous leptin, microinjected into the commissural nucleus tractus solitarii (cNTS), preceding sodium cyanide (NaCN) injection into the in vivo isolated carotid sinus modifies hyperglycemic reflex (HR) and brain glucose retention (BGR). In adult anesthetized (sodium pentobarbital, i.p. 3.3 mg/100 g/saline, Pfizer, Mex), Wistar rats, an in vivo isolated carotid sinus preparation was made in the left carotid sinus. Arterial and venous blood samples were collected from silastic catheters implanted in the carotid artery, jugular sinus and abdominal aorta, before and after leptin injections into cNTS. Exogenous leptin (50 ng/20 nL of aCSF) or leptin vehicle (aCSF 20 nL) were microinjected (stereotaxic infusions) in the cNTS 4 min before NaCN (5 µg/100 g/50 µL saline) or saline (50 µL) injections in the isolated carotid sinus. Leptin before the NaCN injection into the isolated carotid sinus significantly increased the HR and BGR, compared to control rats that only received aCSF and NaCN; when leptin was injected into the cNTS before saline, glucose responses did not vary compared with aCSF plus saline activation experiments. Leptin and its receptors in the cNTS cells probably contribute to their sensitization during hypoxia.

Acknowledgements to Consejo Nacional de Ciencia y Tecnología, México, funding 17704.

The authors confirm that the experiments described here have received ethical approval.
High Fat Feeding in Rats Alters Respiratory Parameters by a Mechanism that is Unlikely to be Mediated by Carotid Body Type I Cells

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The carotid bodies (CB), the primary peripheral chemoreceptors, respond to changes in blood gases with neurotransmitter release, thereby increasing carotid sinus nerve firing frequency and ultimately correcting the pattern of breathing. It has previously been demonstrated that acute application of the adipokine leptin caused perturbations of intracellular calcium and membrane ion movement in isolated CB Type I cells (Pye et al, 2015) and blunted the response of the intact CB to hypoxia (Pye et al, 2016). This study’s aim was to examine, in-vivo, if elevated leptin modulated CB function and breathing.

Rats were fed high-fat chow or control chow for 16-weeks. High-fat fed (HFF) animals gained significantly more weight compared to control fed (CF) animals (n=18; p<.001; 512.56 g ± 14.70 g vs. 444.11 g ± 7.09 g). HFF animals also had significantly higher serum leptin levels compared to CF (n=18; p<.0001; 3.05 ng/mL ± 0.24 ng/mL vs. 1.29 ng/mL ± 0.12 ng/mL). Whole-body plethysmography was used to test the acute hypoxic ventilatory response (HVR) in unrestrained, conscious animals. HFF animals had an attenuated 2nd-phase of the HVR when compared to CF (n=18; p<.05; 710.1 ± 41.9 mL kg⁻¹ min⁻¹ vs. 855.4 ± 44.05 mL kg⁻¹ min⁻¹). CB Type I cells were isolated and intracellular calcium measured; no significant differences in the cellular hypoxic responses between groups were observed.

These data show differences in the 2nd-phase of the HVR caused by high fat feeding are unlikely to be caused by an action of leptin on the Type I cells. However the possibility remains that leptin may have in-vivo postsynaptic effects on the carotid sinus nerve; this remains to be investigated.


This work was funded in part by NIH RO1HL091836

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Increased Day/Night Changes in Hypoxic Ventilatory Response in Chronic Heart Failure

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Respiratory function follows a circadian pattern (Mortola, 2004). Among the mechanisms responsible for these rhythmic changes in breathing include body temperature (Tb) and metabolic rate. Tb and metabolic rate are significant regulators of ventilation, and rhythmic changes in these parameters appear to contribute to day-night variations in ventilation. The carotid bodies also appear to play a role in respiratory circadian rhythms. It has been shown that the threshold for the chemoreflex increases during the inactive phase of the light-dark cycle (Stephenson et al., 2004). Other studies have demonstrated that the isolated rat carotid body shows enhanced responses to hypoxia in the presence of melatonin, a nocturnally released hormone (Chen et al., 2005). Further, melatonin enhances chemoreflex breathing in response to hypoxia in intact animals. Also, chronic hypoxia increases melatonin receptor expression in the carotid body in vitro, thereby increasing the sensitivity of the carotid body to hypoxia in the presence of melatonin. Clinical conditions characterized by chronic or intermittent hypoxemia may also display increased circadian variations in chemoreflex breathing. Chronic heart failure (CHF) is characterized by impaired cardiac output, breathing instability, and thus chronic intermittent hypoxia (Brack et al., 2012). Here, we’ve begun to examine day-night variations in chemoreflex breathing in a rat model of CHF. We predicted that the already heightened chemoreflex responses in CHF would be further exaggerated during the night. We found that both the control group and the CHF animal showed an increase respiratory rate (fR) and minute ventilation (VE) during the night compared to during the day. However, consistent with our hypothesis, the CHF animal also showed a more robust increase in fR during exposure to hypoxic air (10% O2) during the night. Thus, the percent change from day to night in fR was greater in CHF compared to controls.


This project was supported by NIH PO1-HL062222-17

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Abstract No. P12

Effect of Chronic Hypoxia on Pulmonary Hypertension and Endothelial Function in the Rat

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Chronic intermittent hypoxia (CIH) is a determinant factor in the development of the cardiovascular and metabolic effects associated to the obstructive sleep apnea syndrome (OSA) (Sajkov & McEvoy, 2009). Chronic sustained hypoxia (CSH), as found in chronic pulmonary obstructive disease (COPD) or living at high altitude, is involved in the development of pulmonary hypertension (Chaouat et al, 2008).

In this study we used male Wistar rats exposed to CIH (5%O2, 8 h/day, 30 cycles/h 21 days) or CSH (10%O2, 7/14 days), and studied their pulmonary circulation, using measurements of pulmonary arterial pressure (PAP), and contractility and endothelial function in small pulmonary arteries (PA) using myography.

Using in vivo pulmonary catheterism we found that hypertension developed with CSH and CIH, as the PAP went from 11.3±0.8 mmHg (n=5) to 15.5±1.1 (n=6) and 17.7±0.7 (n=6) after 7 and 14 days of CSH, respectively, and to 15.2±0.6 (n=6) in CIH.

Measurements of PA contractility in vitro showed higher responses to 3μM phenylephrine (PE) in CSH 14 days when compared to controls (67.5±8.6 vs 35.8±4.1, as % of 80mM KPSS response, n=10), while slightly increased in CIH (47.3±5.7%, n=8) and CSH 7 days (45.2±5.8%, n=11). Considering endothelial integrity as remaining contraction after 3μM carbachol, we found a light endothelial dysfunction in CIH and CSH 7 days vs. control (22.1±9.1% n=8 and 20.6±7.2% n=9 vs 4.1±2.8% n=10), that was more intense in the CSH 14 days (35.9±5.0%, n=14).

We conclude that CSH and CIH modify PAP in a time dependent way. In vitro results go in the same direction, with 14 days CSH affecting PE contractility and endothelium integrity, while CIH and 7 days CSH don’t affect contraction, and only slightly endothelial function. Effects induced by hypoxic exposure could be due to changes in the NOS expression or altered levels of L-arginine, affecting NO metabolism.


Funded by BFU2015-70616R (MINECO/FEDER) y CIBER CB06/06/0050 (ISCiii)

Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Sexual Dimorphism on the Effects of Chronic Hypoxia in Rat

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Chronic sustained hypoxia (CH) is a situation that can be physiological, living at high altitudes, or pathological in chronic obstructive pulmonary disease. CH produces a sensitization of oxygen sensing structures as CB chemoreceptors, manifested by an increase in hypoxic ventilatory response and sympathetic activity, or in pulmonary vessels resistance, manifested by sustained hypoxic pulmonary vasoconstriction and pulmonary hypertension (PH). Epidemiological studies demonstrate that PH is a predominant disease in young female, suggesting sex-related differences in PH development. Thereby, we have studied the possible sexual dimorphism on the adaptive and pathological effects of CH exposure in male and female rats to a normobaric hypoxic atmosphere (10%O₂; pO₂≈70 mmHg) during 7 (7d) and 14 days (14d).

By plethysmography, we have monitored baseline minute ventilation (MV) and acute hypoxia and hypercapnia responses. No differences were found in MV basal or acute hypoxic test but MV in female rats breathing hypercapnia was higher than in males, at any CH exposure time. CB endogenous catecholamine content (HPLC) at 7d and 14d of CH was higher in male than in female rats, although the rate of synthesis increased similarly in both. Hemodynamics and cardiac effects also were earlier and more intense in female rats, in which pulmonary arterial pressure (PAP) increases from 12.2±0.8 mmHg in controls to 18.0±1.1 mmHg (7 days) and 22.9±1.0 mmHg (14 days) whereas in male rats changes occurred only at 14d of CH (24.8±1.4 vs. 15.2±1.0 mmHg in Control). The female increase correlates with an augmented Fulton Index from 0.29±0.01 to 0.35±0.02 (7d) and 0.35±0.01 (14d) and a diminished heart rate from 414.0±11.4 to 392.9±17.6 (7d) and 259.1±39.8 (14d). These preliminary results suggest a sexual dimorphism in the progression of the hypoxic pulmonary and circulatory effects, to be further investigated.

Supported by grants: BFU2015-70616R (MINECO-FEDER); CIBER CB06/06/0050 (ISCiii)
Protocols approved by Institutional Committee of the University of Valladolid for Animal Care and Use.
Chronic Heart Failure Abolishes Circadian Rhythms in Chemoreflex Breathing

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Respiratory function, including chemoreflex breathing, follows a circadian rhythm (Stephenson, 2000), and the circadian timing system can be altered by abnormal breathing patterns (Jarsky, et al., 2000). Thus, clinical conditions, such as chronic heart failure (CHF), characterized by abnormal respiratory function may show altered circadian rhythms in chemoreflex breathing. In the current study, day-night variations in chemoreflex breathing were examined in a coronary ligation model of CHF in adult male Sprague Dawley rats. Tidal volume, respiratory frequency (fR), and minute ventilation (V̇E) were all determined by whole body plethysmography. Rats were given at least 30 min to acclimate to the chamber and then were exposed to a series of gas challenges (10% O₂, 5% CO₂, 90% O₂, and 5% CO₂ with 90% O₂) with 3-5 min of recovery between each challenge. Chemoreflex breathing was assessed during the day (9am-2pm) and 12 hours later during the night (9pm-2am). Animals were kept on a 12/12 light/dark cycle with lights on at 6am. Data were analyzed using a two way repeated measures ANOVA with gas level and time of day as factors. Based on ex vivo studies from another lab (Chen, 2005), we predicted that the already heightened chemoreflex responses in CHF would be further exaggerated during the night. We found that the control group showed an increase in fR and V̇E during the night under baseline conditions and in response to hypoxia and hypercapnia. However, contrary to our hypothesis, the CHF group failed to show circadian rhythms in either fR or V̇E under any condition, demonstrating that CHF abolishes circadian rhythms in chemoreflex breathing.


This project was supported by NIH PO1-HL062222-17

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Focusing on a Non-Invasive Measurement of Sympathetic Reactivity to Central and Peripheral Chemoreceptor Stimulation in Humans


Department of Physiology and Pharmacology[1], Department of Medicine[2], Department of Community Health Sciences[3], Department of Clinical Neurosciences[4], Cumming School of Medicine, University of Calgary, Alberta, Canada; Faculty of Physical Education and Recreation, University of Alberta, Edmonton Alberta, Canada [5]

BACKGROUND: Sympathetic nervous system (SNS) overactivation is associated with serious cardiorespiratory and metabolic disease. Therefore, incorporating SNS measurements into clinical practice, research and drug discovery is warranted. Current techniques are technically difficult and invasive (microneurography), have poor temporal resolution ([plasma norepinephrine]) or have questionable accuracy (i.e. heart rate variability). The retina of the eye is an outcrop of the brain. Choroid blood vessels, which encase the retina, receive strong sympathetic innervation and can be imaged non-invasively with spectral domain optical coherence tomography (OCT). AIM: Test the association of choroid vascular perfusion density (VPD) to SNS activity in response to respiratory gas challenges and assess if choroid VPD can be used as a surrogate measure of SNS activity. METHODS: OCT images were compared with muscle sympathetic activity (MSNA, microneurography) during periods of induced hypoxia, hyperoxia, hyperoxic-hypercapnia, hyperventilation, end-expiratory breath-hold and, a cold-pressor test in 11 subjects (6 males, 25±2y). RESULTS: Choroid VPD and integrated total MSNA were inversely correlated (R= -0.91; p<0.01) over the 6 manoeuvres. The intra-subject correlation between changes in MSNA and choroid VPD were also strongly and inversely related (R= -0.82±0.04). In contrast the retinal pigment epithelium VPD was unaffected by the cold pressor test and demonstrated responses to respiratory challenges consistent with autoregulation (R=0.54; p=0.27). The repeat measures coefficient of variation for both choroid (5.84±1.60%) and retinal pigment epithelium (4.57±1.57%) VPD were excellent. CONCLUSION: Our data show divergent vascular regulatory mechanisms at play in the retina and choroid in response to respiratory and non-respiratory sympathetic challenges. Choroid VPD provides an accurate readout of SNS activity whereas the retinal pigment epithelium appears to rely on autoregulation. Given OCT imaging is quick and non-invasive, we suggest it may provide a useful method to assess both neurovascular autoregulation and SNS (re)activity.

R.J.A.W. is an Alberta Innovates Senior Scholar and funded by CIHR and NSERC. N.G.J. postdoctoral salary was supported by Alberta Innovates, Canadian Allergy Asthma and Immunology Foundation, and the T Chen Fong Foundation.

Where applicable, the authors confirm that the experiments described here have received ethical approval.
Machiko Shirahata Awardees

Bernardete F. Melo
Erin Leonard
Hortensia Torres-Torrelo
Ian Wenker
Inmaculada Docio
Maria Paz Oyarce
Nicholas Jendzjowsky
Robert Lewis
Ryan Rakoczy
Tara Janes

Machiko Shirahata, M.D., Ph.D.
(1950 - 2016)
In 2016, ISAC members lost a beloved colleague and friend, Machiko Shirahata, M.D., Ph.D. Machiko received her M.D. from Chiba University School of Medicine in Japan in 1985. She did residency training in Anesthesiology and served as the Chief Anesthesiologist at the National Chiba Hospital from 1987-1988. In 1985, she received her Ph.D. from Chiba University in Physiology and Anesthesiology. In 1985, she came to the United States for post-doctoral research training at the University of Pennsylvania in the Department of Physiology. In 1988, she joined the faculty of the Department of Anesthesiology and Critical Care Medicine in the Johns Hopkins University School of Medicine. She subsequently joined the faculty of the Department of Environmental Health Sciences in 1990. During her career, Machiko published over 100 research articles and book chapters. She was an internationally recognized leader in the field of carotid body biology and active member in ISAC for many years. She led the way in defining many critical aspects of the chemosensing properties of the carotid body and its role in a variety of human diseases (sudden infant death, obstructive sleep apnea, hypertension and diabetes). Her love for mentoring trainees in science, and her tireless support of her trainees was unsurpassed. As part of her legacy she wanted to support trainees with interest in hypoxic chemosensitivity to attend the ISAC international meeting. In her honor, Akira Fitzgerald, has made the Machiko Shirahata Trainee ISAC award possible.
In 2015, ISAC lost an amazing person and scientist, Constancio González Martínez, M.D., Ph.D. Constancio, who served as ISAC President 2005-2008, was a superb scientist and dear friend to many, whose work informed so many of us about the carotid body, neurotransmission and oxygen sensing. His remarkable scientific career served as a model to inspire many of us to pursue a similar scientific work.

He was born in 1949 in the village of Renedo de Valderaduey in the Spanish province of León, of which he shared many fond memories and of which he spoke with incredible pride. He received his medical degree in 1974 from the University of Valladolid in Spain. He published his thesis, entitled *Neurotransmission in the Carotid Body* (under Professor Carlos Belmonte) in 1977 and pursued postdoctoral training under Professor Carlos Eyzaguirre at the University of Utah from 1976-1980. Subsequently, Constancio returned to Valladolid where he advanced to the rank of Professor of Physiology, becoming the Director of the Department of Biochemistry and Molecular Biology and Physiology of the Medicine Faculty in 1995, a position he held until his recent death.

Constancio received numerous accolades and awards. He served as President of the Spanish Society of Physiological Sciences and as Editor of *Respiratory Physiology of Neurobiology*. In 2011, he received the prestigious Castilla y León Award for Scientific and Technical Research, awarded in recognition for his teaching and research career and for international contribution to arterial chemoreception knowledge that laid the foundation for understanding other pathologies. He co-authored over 170 articles in international journals, books and monographs. He has published landmark articles on arterial chemoreceptors and state-of-the-art reviews for the Handbook of Physiology, Physiological Reviews and the Neurosciences Encyclopedia. His leadership and vision substantially contributed to the creation of the Institute of Molecular Biology and Genetics (IBGM), a joint research center of the University of Valladolid and the Consejo Superior de Investigaciones Científicas (CSIC).

Many of us will remember him as tireless with an insatiable thirst for knowledge, as a loving husband and father, as a patient teacher and mentor, as a great dancer and as a gracious host who loved his country, his profession and life in general.
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