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Cerebral microvascular blood flow and CO_2 reactivity in pulmonary arterial hypertension☆

Erika Treptow[a,b], Mayron F. Oliveira[a], Aline Soares[a], Roberta P. Ramos[a,b], Luiz Medina[a], Rita Lima[a], Maria Clara Alencar[a], Eloara Vieira Ferreira[a,b], Jaquelina S. Ota-Arakaki[b], Sergio Tufik[c], Luiz E. Nery[a], Lia Rita Bittencourt[c], J. Alberto Neder (MD PhD)[a,b,d,*]

[a] Pulmonary Function and Clinical Exercise Physiology Unit (SEFICE), Respiratory Division, Department of Medicine, Federal University of São Paulo, Paulista School of Medicine (UNIFESP-EPM), Brazil
[b] Division of Respiratory Diseases, Department of Medicine, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil
[c] Departamento de Psicobiologia da Universidade Federal de São Paulo (UNIFESP), Brazil
[d] Laboratory of Clinical Exercise Physiology (LACEP), Division of Respiratory and Critical Care Medicine, Department of Medicine, Queen's University, Kingston, Canada

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ABSTRACT

Hypocapnia and endothelial dysfunction might impair microvascular cerebral blood flow (CBF_{micr}) and cerebrovascular reactivity to CO_2 (CVR_{CO2}). Pulmonary arterial hypertension (PAH) is characteristically associated with chronic alveolar hyperventilation and microvascular endothelial dysfunction. We therefore determined CBF_{micr} (pre-frontal blood flow index (BFI) by the indocyanine green-near infrared spectroscopy methodology) during hypocapnia and hypercapnia in 25 PAH patients and 10 gender- and age-matched controls. Cerebral BFI was lower in patients than controls at similar transcutaneous PCO_2 ($PtcCO_2$) levels in both testing conditions. In fact, while BFI increased from hypocapnia to hypercapnia in all controls, it failed to increase in 17/25 (68%) patients. Thus, BFI increased to a lesser extent from hypo to hypercapnia ("Δ") in patients, i.e., they showed lower Δ BFI/Δ $PtcCO_2$ ratios than controls. In conclusion, CBF_{micr} and CVR_{CO2} are lessened in clinically stable, mildly-impaired patients with PAH. These abnormalities might be associated with relevant clinical outcomes (hyperventilation and dyspnea, cognition, cerebrovascular disease) being potentially amenable to pharmacological treatment.

1. Introduction

The precise regulation of blood flow through the small brain vessels (i.e. pre-capillary arterioles, capillary and glial vessels) is paramount to nutrient and oxygen supply to neurons with important implications for motor-sensory function, neuro-vegetative control and cognition (Ainslie and Duffin, 2009). Owing to anatomic and physiological peculiarities however, cerebral microvascular blood flow (CBF_{micr}) might differ from that observed in large conduit arteries (Zirak et al., 2014). Interrogation of the human cerebral microvasculature may therefore provide a more accurate assessment of actual tissue perfusion than macrovascular hemodynamic measurements (Kuebler et al., 1998; Gora et al., 2002).

The regulation of CBF_{micr} is a sophisticated physiological process that integrates intracranial mediators of cerebral vessel resistance (e.g., cerebral metabolism and auto-regulation) to cardiovascular (e.g., mean blood pressure and cardiac output) and pulmonary gas exchange function (Jordan et al., 2000; Ainslie and Duffin, 2009; Goadsby, 2013). The arterial partial pressure for carbon dioxide ($PaCO_2$) stands out as a key "humoral" regulator of cerebral tissue perfusion as CBF_{micr} increases 3–6% and decreases 1–3% for each mmHg change in CO_2 above and below eupnoeic $PaCO_2$. (Ainslie and Duffin, 2009) Thus, the cerebro(micro)vascular reactivity to CO_2 (CVR_{CO2}) can be measured by relating simultaneous variations in $PaCO_2$ and CBF_{micr}. (Zirak et al., 2014) Of note, endothelial vasodilatory prostanoids, nitric oxide (NO) and the system of phosphodiesterases constitute important mediators of both CBF_{micr} and CVR_{CO2} (Diomedi et al., 2005; Brenner, 2006; Fathi et al., 2011; Pretnar-Oblak, 2014). It is therefore conceivable that those measurements would hold particular relevance to disease condi-

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* Corresponding author at: Division of Respiratory and Critical Care Medicine Queen's University and Kingston General Hospital Richardson House, 102 Stuart Street Kingston, K7L 2V6 ON, Canada.
E-mail address: nederalb@gmail.com (J.A. Neder).

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tions associated with chronic hyperventilation, hypocapnia and impaired NO-mediated microvascular endothelial function.

In this context, pulmonary arterial hypertension (PAH) is a disabling and life-threatening disease in which those specific abnormalities assume a prominent pathophysiological role (Naeije and van de Borne, 2009; Neder et al., 2015) and have negative prognostic implications (Hoeper et al., 2007; Ferreira et al., 2014; Lai et al., 2014). Thus, hypocapnia-induced cerebral vasoconstriction could decrease CBF_{micr} in PAH in addition to low cardiac output (right ventricular dysfunction) (Naeije and Manes, 2014), sympathetic over-activity (Naeije and van de Borne, 2009) and endothelial dysfunction (Pretnar-Oblak, 2014). If this holds true, it is also conceivable that the tonic cerebral vasoconstrictive effects of hypocapnia would contribute to dampen microvascular dynamics relative to $PaCO_2$ variations, i.e., low CVR_{CO2}. In fact, Rosengarten et al. found that PAH patients presented with impaired cerebral microvascular reactivity to visual stimuli which was partially restored when NO bioavailability increased with oral sildenafil. To the best of the authors' knowledge, however, no previous study has measured CBF_{micr} during hypocapnia and hypercapnia thereby allowing the determination of CVR_{CO2} in PAH.

We therefore compared CBF_{micr} (indocyanine green-near infrared spectroscopy methodology) (Kuebler et al., 1998) during hypocapnia and hypercapnia in PAH patients and gender- and age-matched controls. We hypothesized that CBF_{micr} would be lower in both testing conditions in patients than controls; moreover, we anticipated that CBF_{micr} would increase to a lesser extent from hypo to hypercapnia thereby unravelling impaired CVR_{CO2} in PAH. Confirmation of the study hypotheses would not only set the scene for investigations relating impaired CVR_{CO2} to clinically-relevant outcomes but also to test the effects of pharmacological interventions on cerebral microvascular perfusion in PAH.

2. Methods

2.1. Study population

Thirty-one women with right heart catheterization-proved PAH (mean pulmonary artery pressure $\geq$25 mmHg and pulmonary wedge pressure $\leq$15 mmHg) (Galiè et al., 2015) were recruited from the institutional pulmonary vascular clinic. Thirteen age-matched sedentary controls from the general population were also assessed. All patients were under stable medical treatment in the preceding three months. Patients were excluded in the presence of recent disease instability (i.e. worsening of symptoms, syncope, visit to the Emergency Department in the previous month), diagnosis of any other cardiovascular and respiratory diseases, obesity (body mass index >35 kg/m^2 thereby increasing the risk of obesity-hypoventilation syndrome) and inability to perform the CVR_{CO2} protocol. Written informed consent was obtained from all the participants and the study protocol was approved by the Medical Ethics Committee of the Federal University of Sao Paulo, Sao Paulo, Brazil (#16184/12).

2.2. Polysomnography

Whole-night polysomnography was performed using a digital system (EMBLA 7000, Embla System, Inc., Broomfield, CO., USA) in a sleep laboratory, at the usual sleep time. Physiological variables monitored included electroencephalography, electrooculography, electromyography, electrocardiography (ECG), airflow, chest and abdominal respiratory effort through inductance plethysmography, snoring, body position, oxyhemoglobin saturation by pulse oximetry (SpO_2) and heart rate (HR). Respiratory events were classified according to the American Academy of Sleep Medicine's guidelines (Iber et al., 2007).

2.3. Pulmonary function tests

Spirometry was performed using a calibrated pneumotachograph (CPF System™, Medical Graphics Corporation – MGC, St. Paul, MN, USA) according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (Miller et al., 2005). The single breath transfer factor for carbon monoxide (DL_{CO}, mL/min/mmHg) was measured using the 1085D System™ (MGC). Tests were also performed according to ATS/ERS guidelines (Macintyre et al., 2005). Respiratory gases (PO_2 and PCO_2, mmHg)(ABL800 Flex™, Radiometer) were measured prior to the CVR_{CO2} protocol on arterialized (*art*) capillary blood samples obtained from the earlobe after application of a vasodilator cream (Finalgon© Boehringer Ingelheim, Germany).

2.4. CVR_{CO2}

2.4.1. Procedure

Subjects were tested in the afternoon to avoid morning-related decreases in CVR_{CO2} (Ainslie and Duffin, 2009). Upon arrival in the laboratory, the participants rested from 20 min in a silent environment; subsequently, they remained seated while wearing an eye patch to avoid the influence of visual stimulation on CBF_{micr}. Transcutaneous PCO_2 ($PtcCO_2$, mmHg) was continuously measured (see below). The participants breathed through a mouthpiece and a 2-way non-rebreathing valve (2700 series© Hans-Rudolph Inc., Missouri, USA). During the 5-min hypercapnic challenge, valve's inspiratory port was connected to a low resistance tubing circuit leading to a non-diffusing gas bag continuously flushed with 5% CO_2, 21% O_2 and nitrogen balance. During the 5-min hypocapnic challenge, valve's inspiratory port was open to room air and patients hyperventilate following an auditory cue set at the same respiratory frequency recorded in the hypercapnic trial. Care was taken to certify that $PtcCO_2$ reached the minimal target (resting $PtcCO_2$-10 mmHg) within 2 min of hyperventilation. A 10-min in-between test period was observed to allow the physiological variables to return to baseline.

2.4.2. Measurements

In addition to heart rate and respiratory frequency, oxyhemoglobin saturation by pulse oximetry (SpO_2, %) and $PtcCO_2$ (TCM4© Radiometer, Denmark) were continuously measured. $PtcCO_2$ rather than the end-tidal partial pressure for CO_2 ($PETCO_2$) was used to non-invasively estimate $PaCO_2$ due to large and unpredictable $P(a\text{-}ET)CO_2$ differences in patients with pulmonary vascular diseases (Hansen et al., 2007). $PtcCO_2$ electrode was placed on the infraclavicular fossa avoiding large vessels and local musculature After thermal stabilization (44°C), the system baseline reading was changed according to simultaneously-measured $PartCO_2$. Mean $PtcCO_2$ values during the last minute of the trials were recorded for analysis.

Near-infrared spectroscopy (NIRS) (NIRO 200™ Hamamatsu Photonics KK, Japan) was used to obtain an index of CBF_{micr} (cerebral blood flow index, BFI_{cer}) (Kuebler et al., 1998). NIRS measures the passage of the intravenous tracer indocyanine green (ICG) through the tissue microvasculature (Kuebler et al., 1998; Gora et al., 2002; Wagner et al., 2003; Habazettl et al., 2010). The optode was placed on the left forehead close to hair insertion to avoid the paranasal sinuses. Thus, BFI_{cer} provides a semi-quantitative index of pre-frontal, cortical perfusion when brain metabolism (and O_2 consumption) is stable (Wagner et al., 2003). The optode assembly was secured on the skin surface with tape and then covered with an optically dense, black vinyl sheet thus minimizing the intrusion of