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Retinal arteriolar and capillary vascular reactivity in response to isoxic hypercapnia

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ABSTRACT

The aim of the study was to compare the magnitude of vascular reactivity of the retinal *arterioles* in terms of percentage change to that of the retinal *capillaries* using a novel, standardized methodology to provoke isoxic hypercapnia. Ten healthy subjects (mean age 25 years, range 21–31) were recruited. Subjects attended a single visit comprising two study sessions separated by 30 min. Subjects were fitted with a sequential re-breathing circuit connected to a computer-controlled gas blender. Each session consisted of breathing at rest for 10 min (baseline), increase of $P_{ET}CO_2$ (maximum partial pressure of CO_2 during expiration) by 15% above baseline whilst maintaining isoxia for 20 min, and returning to baseline conditions for 10 min. Retinal hemodynamic measurements were performed using the Canon Laser Blood Flowmeter and the Heidelberg Retina Flowmeter in random order across sessions. Retinal *arteriolar* diameter, blood velocity and flow increased by 3.3%, 16.9% and 24.9% ($p < 0.001$), respectively, during isoxic hypercapnia. There was also an increase of *capillary* blood flow of 34.8%, 21.6%, 24.9% ($p \leq 0.006$) at the optic nerve head neuroretinal rim, nasal macula and fovea, respectively. The coefficient of repeatability (COR) was 5% of the average $P_{ET}CO_2$ both at baseline and during isoxic hypercapnia and was 10% and 7% of the average $P_{ET}O_2$ (minimum partial pressure of oxygen at end exhalation), respectively. The overall magnitude of retinal *capillary* vascular reactivity was equivalent to the *arteriolar* vascular reactivity with respect to percentage change of flow. The magnitude of isoxic hypercapnia was repeatable.

1. Introduction

Vascular reactivity is the magnitude of change in retinal hemodynamic measurements to provocative stimuli, such as that elicited by change in arterial blood partial pressure of carbon dioxide ($PaCO_2$) or oxygen (PaO_2). As the measurement of $PaCO_2$ is invasive and painful, the partial pressure of CO_2 in end-tidal gas ($P_{ET}CO_2$) is often used as a surrogate for $PaCO_2$ (Robbins et al., 1990; Ito et al., 2008). Previous research has used various techniques to raise $P_{ET}CO_2$ including the addition of CO_2 to inspired air (Arend et al., 1994; Chung et al., 1999; Dorner et al., 2002; Fallon et al., 1985; Harris et al., 1996; Hosking et al., 2004; Kergoat and Faucher, 1999; Luksch et al., 2002; Pakola and Grunwald, 1993; Roff et al., 1999; Sponsel et al., 1992; Tsacopoulos and David, 1973; Venkataraman et al., 2005b, 2006), and the total re-breathing of exhaled gas (Harino et al., 1995). Each of these methods is associated with a variable concomitant change in end-tidal PO_2 ($P_{ET}O_2$). Inhaling CO_2 results in an unpredictable increase in ventilation and thereby an unpredictable increase in $P_{ET}O_2$ (Rhoades, 2003). Previous studies have failed to consider concomitant changes in PaO_2 during hypercapnia and the derived vascular reactivity values thereby represent change induced by manipulation of PaO_2 as well as $PaCO_2$. In our experience, inducing a 10–15% increase in $P_{ET}CO_2$ by the manual addition of CO_2 to inspired air increased $P_{ET}O_2$ by 13% (Venkataraman et al., 2005b), while $P_{ET}O_2$ was reduced by 6% when using a manually controlled partial re-breathing technique (Venkataraman et al., 2006).

We have developed a partial re-breathing approach that utilizes computer control of inspired gases to achieve consistent changes in $P_{ET}CO_2$ and in $P_{ET}O_2$ in a subject independent of each other and independent of minute ventilation, in order to primarily study retinal and cerebral vascular reactivity (RespirAct™, Thornhill

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Research, Inc., Toronto, Canada). The aim of this study was to compare the percentage change of the magnitude of vascular reactivity of retinal *arterioles* to that of the retinal *capillaries* using a novel, standardized methodology to provoke isoxic hypercapnia. The magnitude of vascular reactivity in response to isoxic hypercapnia is hypothesized to be equivalent in retinal arterioles and capillaries since the retinal circulation is a closed system, i.e. any percentage change in flow in the arterioles would be expected to be similarly reflected in the downstream capillaries. In addition, it is critical to use a repeatable magnitude of $P_{ET}CO_2$ elevation during hypercapnia to reduce the variability in vascular reactivity assessment. A secondary aim therefore was to assess the repeatability of targeted $P_{ET}CO_2$ in normocapnic and hypercapnic phases.

2. Materials and methods

2.1. Sample

The study was approved by the Research Ethics Boards of the University Health Network, University of Toronto, and the Office of Research Ethics, University of Waterloo. All subjects provided signed informed consent prior to agreeing to participate, after explanation of the nature and possible consequences of the study according to the tenets of the Declaration of Helsinki. Ten healthy subjects (8 females) of mean age 25 years (SD 3.2, range 21–31 years) participated in this study. Subjects had corrected visual acuity of 6/6 or better. Exclusion criteria included respiratory disorders, cardiovascular diseases, systemic hypertension, habitual smoking, refractive error greater than ±6.00 DS and ±1.50 DC, any ocular disease, immediate family history of glaucoma, and/or diabetes, and medications with known effects on blood flow (e.g. antihypertensive medication and medication with activity at autonomic receptors, smooth muscles, or those affecting NO release).

2.2. Instrumentation

2.2.1. Gas delivery system

A sequential gas delivery (SGD) breathing circuit (Hi-Ox^{80}, ViasysHealthcare, Yorba Linda CA) was assembled by placing a re-breathing bag on the expiratory port of a commercial three-valve oxygen delivery system. This breathing circuit has been described in detail elsewhere (Gilmore et al., 2005, 2004; Venkataraman et al., 2005b, 2006). The SGD allows subjects to breath exhaled gas (i.e. re-breathe CO_2 enriched gas) when the fresh gas reservoir is depleted (Slessarev et al., 2006).

Algorithms have previously been derived relating subject minute CO_2 production and O_2 consumption, gas flow and composition entering the SGD circuit to values of $P_{ET}CO_2$ and $P_{ET}O_2$ (Slessarev et al., 2007). These algorithms have been set up in a computer-controlled gas blender to provide the gas flow to the SGD (RespirAct™, Thornhill Research, Inc., Toronto, Canada) (Fig. 1). The software allows the user to choose a series of target $P_{ET}CO_2$ and $P_{ET}O_2$ and the duration of each targeted phase.

2.3. Retinal blood flow assessment

2.3.1. Scanning laser Doppler flowmetry of capillaries

The principle and details of the SLDF have been detailed elsewhere (Michelson et al., 1998; Roff et al., 1999; Venkataraman et al., 2005b). It is based on the principle of the Doppler effect. SLDF measurements were undertaken using the Heidelberg Retina Flowmeter (HRF; Heidelberg Engineering GmbH, Dossenheim, Germany, software version 1.03W). The HRF-derived assessment of capillary flow and vascular reactivity represents an indirect measurement of vascular function since it measures the frequency of the intensity oscillation attributable to red blood cell movement in a volume of tissue.

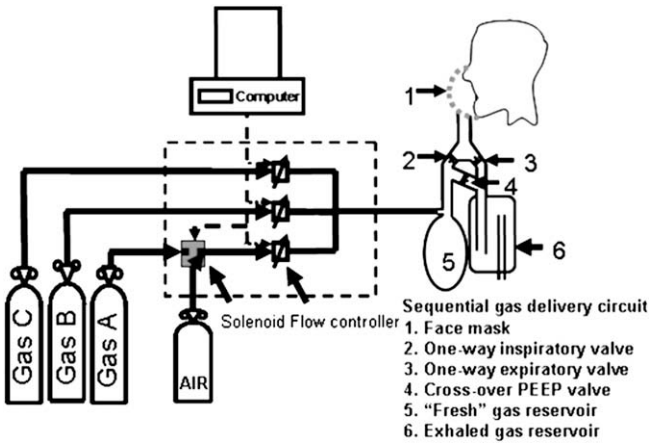

Fig. 1. Schematic representation of the gas flow controller attached to a sequential gas delivery system.

2.3.2. Laser Doppler flowmetry of arterioles

The principle and technical details of the Canon Laser Blood Flowmeter (CLBF; Model 100, Canon, Tokyo, Japan) have been detailed elsewhere (Feke et al., 1987; Gilmore et al., 2005; Guan et al., 2003; Kida et al., 2002; Nagaoka et al., 2002; Riva et al., 1979; Sato et al., 2006; Venkataraman et al., 2006). It is based on the principle of bi-directional laser Doppler velocimetry and simultaneous vessel densitometry. The CLBF simultaneously measures blood velocity (mm/sec) and vessel diameter (μm) to calculate the rate of blood flow (μL/min).

2.3.3. Procedures

Subjects were asked to refrain from caffeine-containing food or drinks for 24 h prior to the study. Subjects were rested for 15 min prior to the start of the breathing paradigm. The study was performed during a single visit using two sessions separated by at least 30 min in order to measure retinal vascular reactivity with both the CLBF and the HRF: the order of use of the instruments was systematically varied between subjects. Each session lasted approximately 40 min.

All participants underwent Heidelberg Retina Tomograph (HRT) imaging before the start of the protocol to determine the difference in the focus between the temporal rim of the optic nerve head (ONH) and the peripapillary retina. This data was then used to establish the optimal focus setting during the HRF imaging of the temporal rim, thereby optimizing the sampling of the anterior ONH capillaries (Sehi and Flanagan, 2004). The HRF focus and sensitivity settings were maintained constant during all phases of the breathing paradigm.

The mask of the breathing circuit was sealed to the face using adhesive tape (Tegaderm, 3M Health Care, St Paul, MN, USA). Initially airflow was set to exceed minute ventilation and thus prevent re-breathing. Tidal gas concentrations were monitored continuously. When $P_{ET}CO_2$ became stable (less than 2 mmHg change over 2 min), end-tidal and mixed exhaled O_2 and CO_2 partial pressures were calculated from gas sampled from the face mask and the exhaled gas reservoir, respectively. CO_2 production was calculated automatically by the computer-controlled gas blender as the product of airflow entering the breathing circuit and the mixed expired concentrations of CO_2 (Jones, 1984; Pennock and Donohoe, 1993). O_2 consumption was calculated as the product of airflow and the difference in O_2 concentration in air and mixed exhaled gas. Three gas stages were implemented at each session. In the first