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Does respiratory drive modify the cerebral vascular response to changes in end-tidal carbon dioxide?

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Running title: CVR and respiration

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New Findings

What is the central question of this study?

An interaction exists between the regulatory systems of respiration and cerebral blood flow (CBF), because of the same mediator (carbon dioxide, CO_2) for both physiological systems. The present study examined whether the traditional method for determining cerebrovascular reactivity to CO_2 (cerebrovascular reactivity; CVR) is modified by changes in respiration.

What is the main finding and its importance?

CVR was modified by voluntary changes in respiration during hypercapnia. This finding suggests that an alteration in the respiratory system may under- or over-estimate CVR determined by traditional methods in healthy adults.

ABSTRACT

The cerebral vasculature is sensitive to changes in the arterial partial pressure of carbon dioxide (CO_2). This physiological mechanism has been well established as a cerebrovascular reactivity to CO_2 (CVR). However, arterial CO_2 may not be an independent variable in the traditional method to assess CVR since the cerebral blood flow (CBF) response is partly affected by the activation of respiratory drive or higher centers in the brain. We hypothesized that CVR is modified by changes in respiration. To test our hypothesis, in the present study, ten young healthy subjects performed hyper- or hypo-ventilation to change end-tidal CO_2 ($P_{ET}CO_2$) under different concentrations of CO_2 gas inhalation (0, 2.0, 3.5%). We measured middle cerebral artery mean blood flow velocity (MCA *V*m) by transcranial Doppler to identify the CBF response to change in $P_{ET}CO_2$ during each condition. At each F_ICO_2 condition, $P_{ET}CO_2$ was significantly altered by changes in ventilation, and MCA *V*m changed accordingly. However, the relationship between changes in MCV *V*m and $P_{ET}CO_2$ as a response curve of CVR was reset upwards and downwards by hypo- and hyper-ventilation, respectively, compared with CVR during normal-ventilation. The findings of the present study may provide the possibility that an

alteration in respiration under- or over-estimates CVR determined by the traditional methods.

Key words: respiratory, ventilation, central respiratory chemoreflex, higher center drive, cerebral blood flow

Introduction

The cerebral vasculature is sensitive to changes in the arterial partial pressure of carbon dioxide (CO_2) (Kety & Schmidt, 1946, 1948a, b; Markwalder *et al.*, 1984). This physiological response of the cerebral vasculature is termed "cerebrovascular reactivity to CO_2 (CVR)." For example, patients with cerebral vascular disease often present with an attenuated CVR (Markus & Cullinane, 2001; Glodzik *et al.*, 2013; Richiardi *et al.*, 2015), indicating that the CVR plays an important role in cerebral blood flow (CBF) regulation. In investigations regarding the cerebral circulation, therefore, many researchers have measured CVR as one of the key CBF regulatory mechanisms.

The method of determining CVR has been well established, and the CVR is identified by the response of CBF to the change in arterial partial pressure of CO_2, which is manipulated by changes in end-tidal CO_2 in inspiratory gas (hypercapnia) and hyper-ventilation (hypocapnia)(Markwalder *et al.*, 1984). Importantly, this methodology is based on the basic concept that CBF responds primarily to changes in CO_2 with little influence of other physiological factors. However, it has been suggested that several other physiological factors in combination with arterial CO_2 concentration, such as respiration, also contributes to a change in CBF at rest (Ogoh & Ainslie, 2009a, b).

Respiratory disease such as sleep apnea, or acute and chronic hypoxia alters CBF regulation (Ogoh *et al.*, 2013; Ogoh *et al.*, 2014; Ponsaing *et al.*, 2018), suggesting that alteration in the function of the respiratory system modifies the cerebral circulation. Indeed, our previous findings (Ogoh *et al.*, 2008; Ogoh *et al.*, 2009) demonstrated that an interaction exists between the regulatory systems of respiration and CBF, because the

same mediator (CO_2) regulates both the central respiratory chemoreflex and CVR. For example, in the traditional method to determine CVR with hypercapnia, the *operating point* of arterial CO_2 tension ($P_{ET}CO_2$) should be due to the balance between respiratory gas CO_2 concentration and any increase in ventilation (respiratory drive) via activation of the central respiratory chemoreflex. Also, by using hypocapnia, voluntary hyperventilation is caused by the activation of higher centers in the brain (central neural drive, which is additive to the chemoreflex drive to breathe) that may affect both cerebral vasculature and the respiratory system via increases in sympathetic nervous activity. Therefore, the traditional method for determining CVR via changes in CO_2 modifies the respiratory system, which may indirectly affect the CBF response to changes in CO_2. Therefore, under hypercapnia or hypocapnia conditions, it is possible that $PaCO_2$ may not be an independent variable to determine traditional CVR, as the CBF response is affected partly by activation of respiratory drive or higher centers in the brain. However, no study investigated the effect of respiration on CVR determination regarding the traditionally used methods of hyper- and hypocapnia. Under these backgrounds, we hypothesized that CVR is modified by changes in respiration. To test our hypothesis, in the present study, we measured the CVR response to changes in ventilation (hyper- or hypo-ventilation) under stepwise steady-state CO_2 gas concentrations.

Methods

Ethical approval

The present study was approved by the Human Subjects Committee of Morinomiya University of Medical Sciences (No. 2018-057). All procedures in the present study conformed to the standards set by the *Declaration of Helsinki*. This study was not registered in a database. Each subject provided written informed consent to participate in the present study.

Participants

Ten healthy subjects (Nine men and one female; aged 20.9 ± 1.0 years, height 169.2 ± 5.9 cm, weight 64.5 ± 8.3 kg, mean ± SD) were recruited to participate in the study. All subjects were free of any known cardiovascular and pulmonary disorders, and were not using prescribed or over the counter medications. Before the experiment, each subject

visited the laboratory for familiarization with the techniques and procedures. When visiting the laboratory, subjects were requested to abstain from caffeinated beverages for 12 h and strenuous physical activity and alcohol for at least 24 h before the day of the experiment.

Measurements

All studies were performed at a constant room temperature between 23 and 24°C with external stimuli minimized. Heart rate (HR) was monitored using a lead II electrocardiogram (ECG), and measured using a cardiotachometer (AT601G, Nihon Kohden, Tokyo, Japan) triggered by the R wave on the electrocardiogram. Blood pressure was measured at 1-min intervals with an automatic manometer placed over the left radial artery (EBP-300, Minato Medical Science Co., LTD, Osaka, JAPAN) at 1-min intervals. The middle cerebral artery blood velocity (MCA V) was obtained continuously by transcranial Doppler ultrasonography (DWL Doppler Box-X; Compumedics, Germany). A 2 MHz Doppler probe was placed over the right temporal window and fixed with an adjustable headband and adhesive ultrasonic gel. Ventilatory responses were measured using an open-circuit apparatus (model 8250, Hans Rudolf). The subject breathed through a face mask attached to a low-resistance one-way valve with a flow meter (ARCO2000-MET, Arcosystem, Chiba, Japan). The valve mechanism allowed subjects to inspire room air or a selected gas mixture from a 200 l Douglas bag containing 0.0, 2.0 or 3.5% CO_2 in 21% O_2 with nitrogen (N_2) balance. The total instrumental dead space was 200 ml. Respiratory and metabolic data during the experiments were recorded by an automatic breath-by-breath respiratory gas-analyzing system consisting of a differential pressure transducer, sampling tube, filter, suction pump and mass spectrometer (ARCO2000-MET, Arco System, Chiba, Japan). Oxygen and CO_2 measurements were calibrated using a standard gas of known concentrations before each test. We digitized expired flow, CO_2 and O_2 concentrations, and derived tidal volume (V_T), minute ventilation (V_E), end-tidal O_2 ($P_{ET}O_2$) and end-tidal CO_2 ($P_{ET}CO_2$). Flow signals were converted to single breath data by matching to gas concentrations identified as single breaths using $P_{ET}CO_2$, after accounting for the time lag (350 ms) in gas concentration measurements. The corresponding O_2 uptake and CO_2 output values for each breath were calculated from inspired-expired gas concentration differences, and by expired ventilation (V_E), with inspired ventilation being calculated by N_2 correction.

Experimental protocol

Before the experiment day, each subject underwent the same experiment procedures as those used during the main experimental day to ensure familiarization with the experimental protocols. On the experimental day, subjects arrived at the laboratory at least 2 h after a light meal. The present study consisted of two protocols (Protocol 1 and 2, Fig. 1).

PROTOCOL 1 (normal protocol for identifying CVR to hypercapnia condition)

First, all subjects underwent Protocol 1 (Fig. 1), and rested in an upright position in a comfortable chair following instrumentation. Five minutes of baseline data were recorded whilst the subjects breathed room air, wearing a face mask. The mean MCA *V* (MCA *V*m) response to hypercapnia was induced by the step change in the fraction of inspired CO_2 (F_ICO_2, 0.00, 0.02, 0.035). In order to permit CO_2 at the central respiratory chemoreceptors to reach a new steady-state, each F_ICO_2 trial ran for 8 min which is sufficient in previous trials (Honda *et al.*, 1983).

PROTOCOL 2 (main study)

Similarly, in Protocol 2, the MCA *V*m response to hypercapnia was induced by the step change in F_ICO_2 (0.00, 0.02, 0.035). Each F_ICO_2 trial ran for 12 min. During each CO_2 condition, the change in ventilation (hyper- and hypo-ventilation) was used to manipulate $P_{ET}CO_2$. To avoid the possible effects of different breathing patterns on the V_E-$P_{ET}CO_2$ relationship, in the hyperventilation trials, both V_T and breathing frequency were altered deliberately by matching the breathing pattern to that recorded during hypercapnia trials. In the hypoventilation trial, V_E was set to 80% of the steady-state value during the 0.00 F_ICO_2 trial (i.e. during spontaneous breathing). The breathing pattern was estimated from the relationship between V_E and V_T in each subject.

During hypo- and hyper-ventilation trials, the inspired and expired volume curves were continuously displayed on a screen monitor. Visual and audio signals were constructed from the breathing pattern of the subjects during the hypercapnia trials. The target V_T level was simultaneously displayed on the same screen monitor in each trial. The subjects were instructed to match their volume curve with the target V_T level, and to breathe according to the sound of the metronome. As a result, both the V_T and breathing

frequency, and thus V_E, were precisely controlled with visual and audio feedback specifically for each individual.

Data processing

Signals from the mass spectrometer, flow sensor, pressure transducer, cardio-tachometer and transcranial Doppler ultrasonography were synchronized on-line using a personal computer, and displayed continuously during all experiments. The signals were logged at 200 Hz using a 12-bit analog-to-digital converter (AD12-8(PM) CONTEC Co., Ltd, Osaka, Japan). The analytical software program is custom-made by our laboratory. Measurements variables, HR, V_E, V_T, $P_{ET}O_2$, $P_{ET}CO_2$ and MCA *V*m, were averaged over 30s at the end of each stage in Protocol 1 and 2. Mean blood pressure (MBP) was averaged from two time points within each stage.

Statistical Analysis

Data were analyzed using the Statistics Package for Social Scientists (IBM SPSS Statistics Version 22.0). Differences between values were evaluated using a two-way repeated-measures analysis of variance (ANOVA, gas concentration<0, 2.0 and 3.5%> × respiration<hypo-, normo-, hyper-ventilation>) followed by a Student - Newman - Keuls post-hoc tests. Pearson's correlation coefficient evaluated the relationship between relative changes in V_E and $P_{ET}CO_2$. A $P < 0.05$ denoted statistical significance for all two-tailed tests.

Results

In the present study, subjects voluntarily changed ventilation to manipulate $P_{ET}CO_2$ under stepwise inhalation of CO_2 gas concentrations (0, 2.0 and 3.5%). In order to manipulate ventilation, each subject changed respiratory rate and V_T, according to his or her individual breathing pattern obtained with regular breathing. Expectedly, all subjects were able to change their V_E to the modified target during each condition e.g. hypercapnia (Table 1 and Fig. 2). Subsequently, $P_{ET}CO_2$ was manipulated by the change in inhaled CO_2 gas concentration and/or V_E (Fig. 3).

The change in $P_{ET}CO_2$ during hyper-, normo-, hypo-ventilation did not effect HR (P=0.511, Table 1) but did change MAP (P=0.041, Table 1). Regarding the response of

MCA *V*m to the change in $P_{ET}CO_2$, the normo-ventilation CVR in protocol 2 was similar with the CVR in protocol 1 (Fig 4A). However, from baseline (norm-ventilation condition), the CVR response (the relationship between $P_{ET}CO_2$ and MCA *V*m) was reset upwards and downwards by hypoventilation and hyperventilation, respectively (Fig 4B) without any change in the slope of the linear relationship between $P_{ET}CO_2$ and MCA *V*m (P=0.301, Fig. 4C).

Discussion

One novel finding of the present study was that CVR, determined using the traditional method, was modified by voluntary changes in respiration during hypercapnia. The findings of the present study provide the possibility that an alteration in the respiratory system may underestimate or overestimate CVR determined by traditional methods in healthy adults.

The concept of the traditional method for determining CVR is that arterial CO_2 concentration, e.g. $P_{ET}CO_2$, $PaCO_2$, is an independent variable that determine changes in CBF. At least, it is thought that CO_2 is a much stronger mediator of CBF compared with other physiological factors. For example, arterial blood pressure has a limit to contribute to changes in CBF because of cerebral autoregulation (Lassen, 1959). Therefore, it was expected that using the traditional CVR method, even ventilation-induced change in CO_2 as well as inspiratory CO_2 gas can identify characteristics of CBF regulation on one linear response CVR curve. For example, the operating point of CVR should be moving along its response curve by a respiratory-mediated alteration in CO_2 (grey arrows, Fig. 5A). Our data of the present study clearly demonstrated that the response of CBF to hyper- or hypo-ventilation induced-change in $P_{ET}CO_2$ was likely similar to the relationship between inspiratory CO_2 gas-induced change in $P_{ET}CO_2$ and CBF under the hypercapnia condition (Slope, P=0.301, Fig 4B and C). However, in contrast to this traditional concept, the operating point of CVR did not follow the typical response curve during hypo- or hyper-ventilation (black arrow, Fig. 5A). In other words, the CVR response curve shifted upwards or downwards by hypo-or hyper-ventilation, respectively. These findings mean that hypercapnia evokes hyperventilation via the central respiratory chemoreflex, and consequently it moves the operating point of CVR downwards outside of its typical response curve. In contrast, this response

(hyperventilation) does not occur during normocapnia because there is no central respiratory chemoreflex (no change in ventilation) (Fig 5B). Taken together, the traditional method for determining CVR does not maintain resting ventilation in healthy subjects because of the 5-6% stimulation of hypercapnia. Therefore, our findings suggest that the respiratory response to CO_2 may modify the CVR determined by the traditional method, that may under- or overestimate actual cerebrovascular responses to CO_2. Also, an increase in MAP was observed during the normocapnia hypoventilation condition (Table 1). The change in perfusion pressure may also modify the CVR response, and hence the change in perfusion pressure may be another limitation for the traditional methods to determine CVR. In the present study, the effect of changes in MAP on CBF could not be identified, thus, it is unclear whether CBF is independent from changes in perfusion pressure using this method. Further investigations are needed to identify the effect of changes in perfusion pressure on CVR. An important point of the present study is that, regardless of change in MAP, the operating point of CVR did not follow the typical response curve during hypo- and hyperventilation. However, the physiological mechanism responsible for the changes in ventilation-induced shift of the CVR response curve is unknown from the data in the present study.

Previous studies suggest that the respiratory system partly plays a role in CBF regulation (Ogoh *et al.*, 2008; Ogoh *et al.*, 2009). Importantly, both of these systems are sensitive to the same mediator, i.e., CO_2, at a set point. Therefore, it is physiologically plausible that these systems are closely linked (Ogoh, 2019). The CBF response to hypercapnia was inversely related to the increase in ventilation, indicating that a lower CVR may result in less CO_2 washout and consequently causes greater ventilatory stimulation via a central respiratory chemoreflex (Peebles *et al.*, 2007). Also, some previous studies (Xie *et al.*, 2006; Cummings *et al.*, 2007) reported that changes in CVR affect stability of the ventilatory responsiveness to CO_2 via alterations in the degree of washout in central chemoreceptor hydrogen [H^+]. Clinically, respiratory disease such as sleep apnea or chronic hypoxia modifies CBF regulation (Ogoh *et al.*, 2013; Ogoh *et al.*, 2014; Ponsaing *et al.*, 2018). Moreover, patients with congenital central hypoventilation syndrome (CCHS) lack chemoreceptor control of breathing and seriously hypoventilate during non-rapid eye movement (non-REM) sleep. Many CCHS subjects breathe adequately during many waking behaviors associated with arousal, cognitive activity or

exercise (Shea, 1997). However, in these patients CBF regulation is impaired, and subsequently results in abnormal cerebral blood oxygenation and volume changes to CO_2 and O_2 challenges (Macey *et al.*, 2003; Macey *et al.*, 2010). The CCHS patients showed 28% higher overall CBF, and this may relate to high CO_2 levels (Macey *et al.*, 2010). Taken together, it is reasonable to physiologically suggest that the change in ventilation via central respiratory chemoreflex modifies the response of CBF in order to maintain an adequate arterial CO_2 concentration for brain homeostasis.

One important aspect regarding our results is that traditional CVR using hyperventilation-induced hypocapnia includes the influence of central drive (respiratory drive), which modifies the CBF response to changes in arterial CO_2 concentration. On the other hand, hypercapnia stimulation with the traditional CVR method does not include higher central drive (respiratory drive) because hypercapnia is caused by an increase in respiratory CO_2 gas concentration. However, the high hypercapnia condition activates central chemoreceptors and subsequently causes hyperventilation as well as hypertension (Table 1). In contrast, a mild hypercapnia ($FiCO_2$ 2.00) did not cause hyperventilation, indicating that the mild hypercapnia condition did not activate the central chemoreflex. Using the traditional method for determining CVR, therefore, arterial CO_2 concentration may not be an independent variable to determine CBF. This is because hypo-, normo- and hyper-capnia conditions includes different physiological factors (higher central drive, central chemoreflex or arterial blood pressure) that may exert separate influences on the cerebral vasculature. These findings also suggest that it may be necessary to distinguish between the CBF response determined by hypocapnia and hypercapnia as a part of complete CVR profile.

Moreover, we may need to carefully consider the limitations of the traditional method and evaluation for determining CVR, to avoid an under- or overestimation in the CVR characteristic. For example, it takes ~ 7 min to reach a steady-state of the response of V_E to the change in arterial CO_2 (Honda *et al.*, 1983). However, most previous studies use only 3-4 min of CO_2 stimulation to identify the CBF response for determining CVR, because the CBF response to CO_2 manipulation is quick. If respiration modifies the CVR, this protocol (3-4 min) may be too short to identify a complete CVR profile because of the lack of time for an attainment of respiratory steady-state. To identify an exact CVR, the

response of CBF to change in CO_2 may need to be evaluated during steady-state ventilation in order to reduce the influence of changes in respiration on CBF.

Clinical Implications

Our findings may also have clinical implications. For example, an attenuation in CVR in patients with respiratory disease (Hartmann *et al.*, 2012; Lewis *et al.*, 2019) may not be due to only alterations in the cerebral vasculature. CVR may be associated with the respiratory system directly as well as an impaired cerebral vasculature response to changes in arterial CO_2. Therefore, it may be difficult to compare CVR determined by the traditional method simply between healthy subjects and patients with respiratory disease.

Limitations

In the methods determining CVR using $P_{ET}CO_2$, it is based on the notion that $P_{ET}CO_2$ is closely related to $PaCO_2$. Although the $P_{ET}CO_2$ estimate of $PaCO_2$ may have limitations in this study, our previous studies revealed that $P_{ET}CO_2$ was tightly correlated with $PaCO_2$ throughout the hypercapnic and hypocapnic range under hypercapnia, and hyper- and hypo-ventilation conditions across various experimental conditions (Miyamoto *et al.*, 2014). Therefore, it seems that the change in $P_{ET}CO_2$ observed during these experimental conditions likely reflect changes in $PaCO_2$. On the other hand, Robbins et al. (Robbins *et al.*, 1990) reported that $P_{ET}CO_2$ was shown to consistently underestimate $PaCO_2$, a finding that is consistent with our literature (Miyamoto *et al.*, 2014; Miyamoto *et al.*, 2015). Jones et al. (Jones *et al.*, 1979) reported that $P_{ET}CO_2$ is higher than $PaCO_2$ when F_ICO_2 are increased, whereas it is lower under normal conditions. Therefore, the values of cerebral CO_2 reactivity (CVR) may be underestimated using $P_{ET}CO_2$ as opposed to $PaCO_2$. Second, the visual feedback method made it possible to control accurately both the VT and breathing frequency, and thus V_E. However, the inspiration and expiration duration during the CVR trials were not controlled. Therefore, this non-physiological respiration pattern might have also affected the $P_{ET}CO_2$ response to V_E. However, we have examined the CBF response to change in ventilation (hyper- or hypo-ventilation) under different inhaled CO_2 gas concentrations. Thus, our interpretation of the observed differences in CBF in each experimental condition is likely to be valid.

Conclusion

In the present study, we successfully manipulated V_E during hypercapnia via control of respiratory rate and V_T. Importantly, this was according to each individual's respiratory pattern. Under hyper- or hypo-ventilation, the relationship between change in $P_{ET}CO_2$ via respiratory CO_2 gas and CBF was unchanged from that during normal breathing. However, the response of CBF to hyper- or hypo-ventilation induced-change in $P_{ET}CO_2$ was different from that caused by only changes in inspiratory CO_2 gas concentration. Thus, it is likely that the upwards and downwards shift in CVR by hypo- and hyperventilation, respectively. Since respiration modifies the CVR function curve, the stability of respiration may be important to determine CVR with the traditional methods.

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Additional information

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

Author contributions: S.O., T.M. conception and design of research; S.O., K.S., T.W., K.T., S.S., G.I. performed experiments; S.O., K.S., T.M. analyzed data; S.O., K.S., T.M. interpreted results of experiments; S.O. prepared figures; S.O., T.B., S.S., T.M. drafted manuscript; all authors edited and revised manuscript; all authors approved final version of manuscript.

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Table 1. Respiratory and hemodynamic response to change in the fraction of inspired CO_2 (F_ICO_2) and respiration.

$FiCO_2$		MCA Vm (cm/s)	V_E (l/min)	RR (/min)	V_T (ml)	$P_{ET}CO_2$ (mmHg)	$P_{ET}O_2$ (mmHg)	MAP (mmHg)	HR (bpm)
0	**Hypo**	65 ± 14	10.4±3.9*#$	13.2±4.5	862±353	39±5	103±11	100±5#	66±15
	Norm	57 ± 14	15.6±8.1*	16.0±6.5	1012±298	37±6	110±10	96±7	62±10
	Hyper	47 ± 10	16.9±6.5*	15.7±5.1	1180±586	34±5	115±8	92±12*	71±13
0.02	**Hypo**	63 ± 15	13.0±4.5#$	14.3±6.0	961±264	42±5	124±7	97±7	61±13
	Norm	56 ± 15	15.8±4.2*#	15.8±5.1	1086±285	40±4	131±7	99±9	64±8
	Hyper	51± 18	19.5±4.3*	16.8±4.7	1222±343	38±3	136±4	94±7*	68±13
0.035	**Hypo**	69 ± 20	15.2±3.8#$	16.4±6.0	988±246	46±2	124±9	99±7	73±35
	Norm	63 ± 17	22.3±2.9#	17.6±4.8	1366±408	44±3	132±8	97±6	66±10
	Hyper	54 ± 14	26.8±4.4	18.9±5.5	1533±511	42±3	136±7	102±7	69±11
P-Value	**Respiration**	P<0.001	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001	P=0.284	P=0.455
	CO_2	P=0.133	P<0.001	P=0.002	P=0.002	P<0.001	P<0.001	P=0.291	P=0.418
	Interaction	P=0.840	P=0.025	P=0.804	P=0.125	P=0.854	P=0.966	P=0.041	P=0.566

Values are means ± SD. hypo, hypoventilation; norm, normoventilation; hyper, hyperventilation; MCA Vm, middle cerebral artery mean blood velocity; V_E, ventilation; RR, respiratory rate; V_T, tidal volume; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $P_{ET}O_2$; end-tidal partial pressure of oxygen, MAP, mean arterial pressure; HR, heart rate. *Different from 3.5%, P<0.05 # Different from hyperventilation, P<0.05, $ Different from normoventilation, P<0.05.

Figure Legends

Figure 1. Experimental protocol 1 and protocol 2.

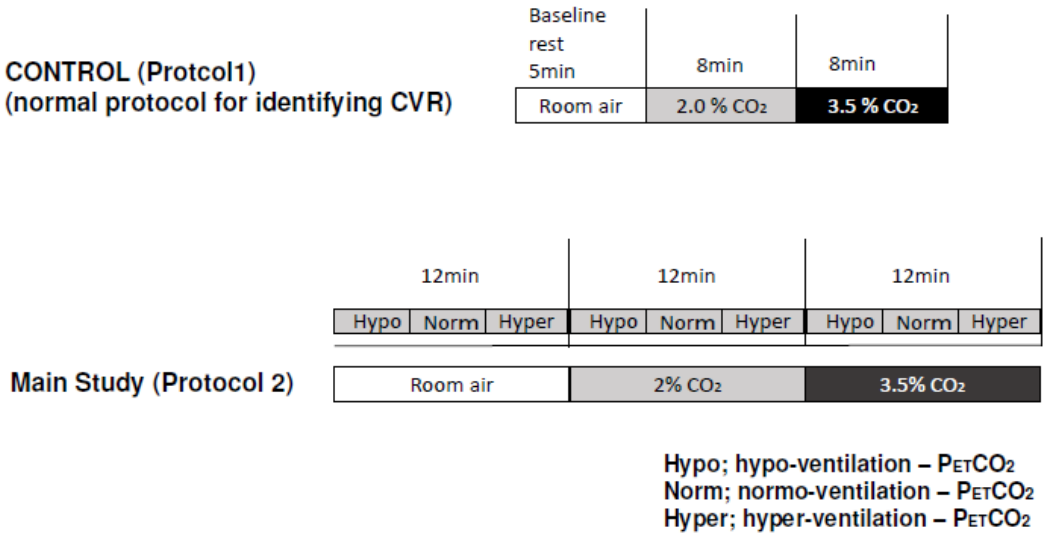

Figure 1

Figure 2. Change in ventilation by hypo- (Hypo), normo- (Norm), hyper-ventilation (Hyper) under hypercapnia condition (0, 2, 3.5% CO_2). Values are means ± SE. *Different from 3.5%, P<0.05 # Different from hyperventilation, P<0.05, $ Different from normoventilation, P<0.05.

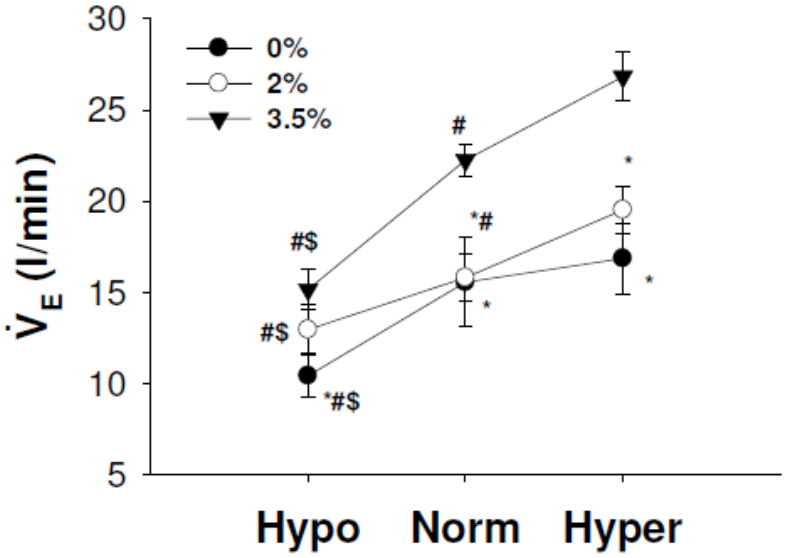

Figure 2

Figure 3. Change in end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) by ventilation (hypo-, normo-, hyper-ventilation; Hypo, Norm, Hyper) and inspiratory CO_2 gas concentration (0, 2, 3.5%). Values are means ± SE.

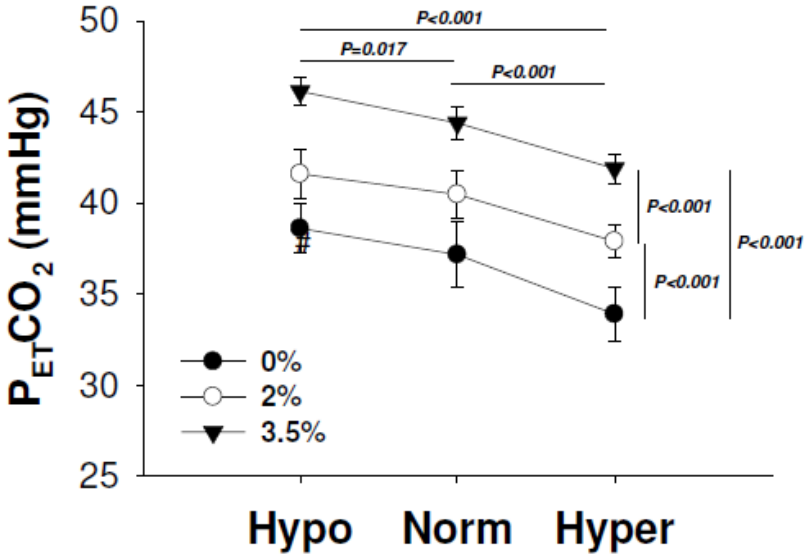

Figure 3

Figure 4. A, The response of middle cerebral artery mean blood velocity (MCA *V*m) to change in end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) during norm-ventilation (free-breathing) in protocol 1 and 2; B, The response of MCA *V*m to change in $P_{ET}CO_2$ during hypo- (Hypo), normo- (Norm) and hyper-ventilation (Hyper) in Protocol 2; C, the slope of the liner relationship between $P_{ET}CO_2$ and MCA *V*m during Hypo, Norm and Hyper. Values are means ± SE.

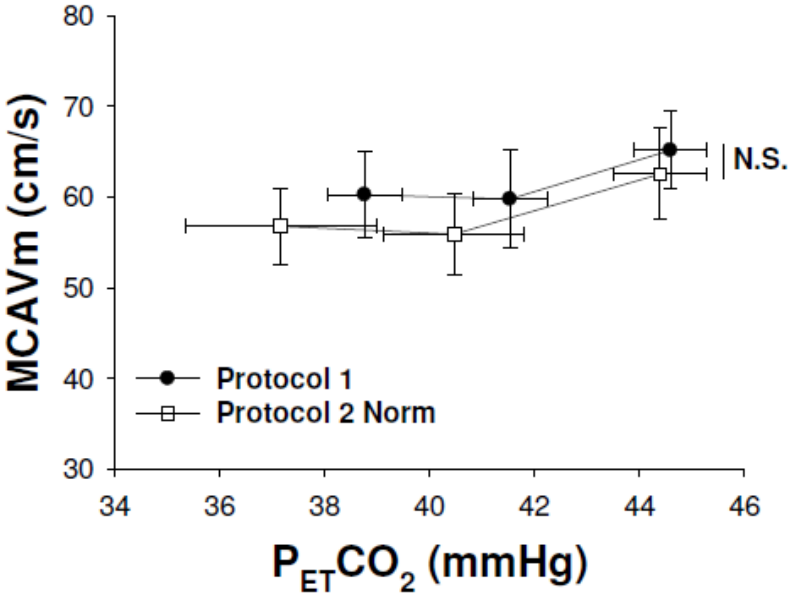

Figure 4A

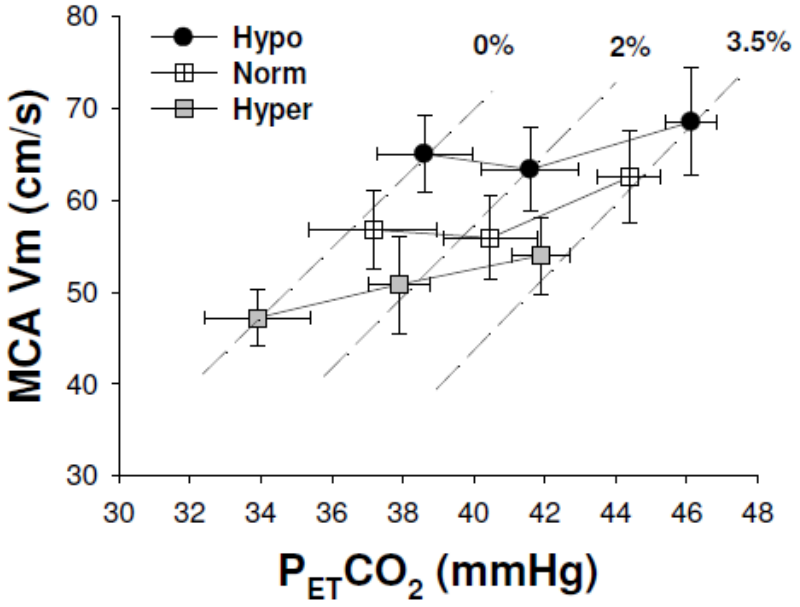

Figure 4B

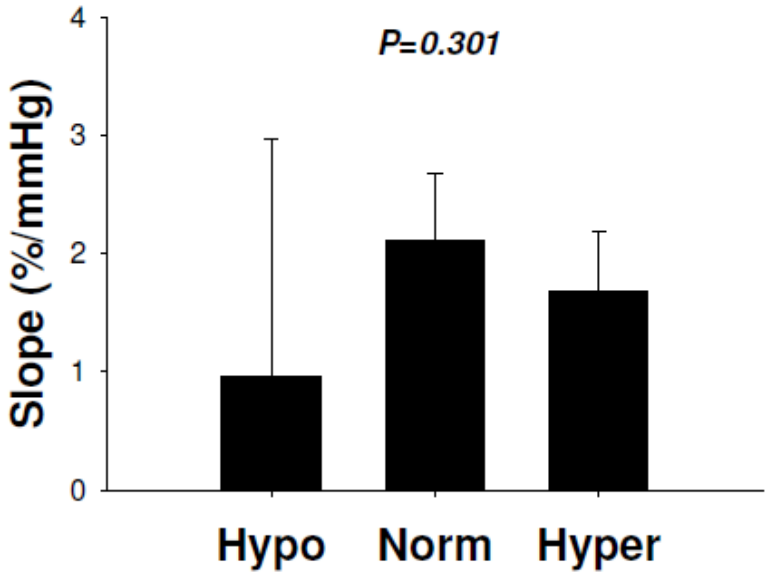

Figure 4C

Figure 5. A, In the concept of CVR, the operating point of CVR should move along the response curve via respiratory alteration-induced changes in CO_2 (grey arrow). In the present study, the operating point shifted from its normal response curve during hypo- or hyperventilation (black arrow); B, In the traditional method of determining CVR, high CO_2 stimulates the central respiratory chemoreflex, and subsequently causes hyperventilation. Thus, the hypercapnia condition shifts the operating point of CVR downwards, outside of the normal (typical) response curve observed during normocapnia (baseline).

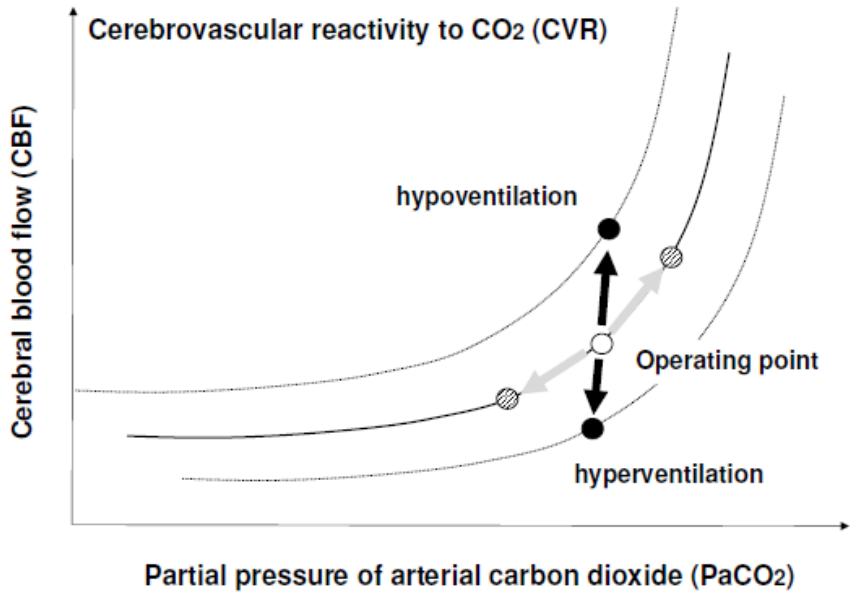

Figure 5A

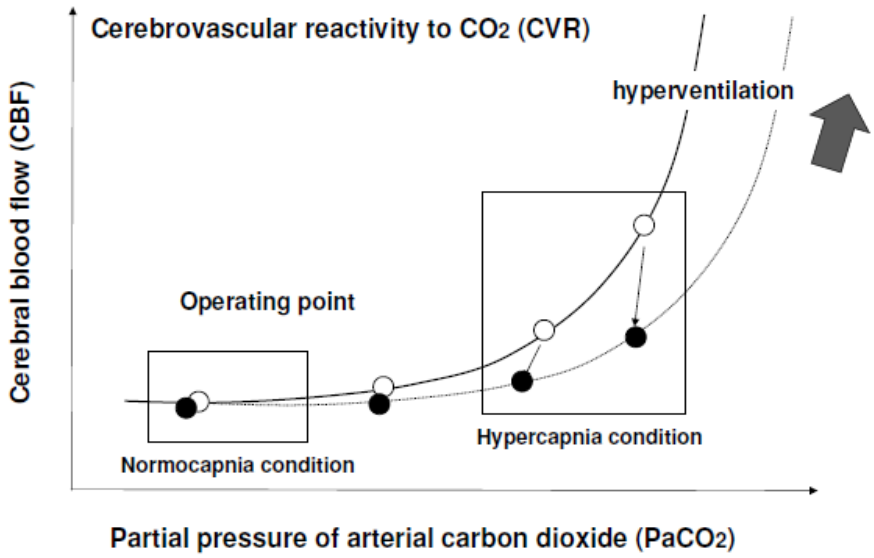

Figure 5B