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Sympathetic regulation of the human cerebrovascular response to carbon dioxide

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Peebles KC, Ball OG, MacRae BA, Horsman HM, Tzeng YC. Sympathetic regulation of the human cerebrovascular response to carbon dioxide. *J Appl Physiol* 113: 700–706, 2012. First published June 28, 2012; doi:10.1152/japplphysiol.00614.2012.—Although the cerebrovasculature is known to be exquisitely sensitive to CO_2, there is no consensus on whether the sympathetic nervous system plays a role in regulating cerebrovascular responses to changes in arterial CO_2. To address this question, we investigated human cerebrovascular CO_2 reactivity in healthy participants randomly assigned to the α_1-adrenoreceptor blockade group (9 participants; oral prazosin, 0.05 mg/kg) or the placebo control (9 participants) group. We recorded mean arterial blood pressure (MAP), heart rate (HR), mean middle cerebral artery flow velocity ($MCA_{V\ mean}$), and partial pressure of end-tidal CO_2 ($P_{ET_{CO_2}}$) during 5% CO_2 inhalation and voluntary hyperventilation. CO_2 reactivity was quantified as the slope of the linear relationship between breath-to-breath $P_{ET_{CO_2}}$ and the average $MCAv_{mean}$ within successive breathes after accounting for MAP as a covariate. Prazosin did not alter resting HR, $P_{ET_{CO_2}}$, MAP, or $MCA_{V\ mean}$. The reduction in hypocapnic CO_2 reactivity following prazosin (-0.48 ± 0.093 cm·s^{-1}·mmHg^{-1}) was greater compared with placebo (-0.19 ± 0.087 cm·s^{-1}·mmHg^{-1}; $P < 0.05$ for interaction). In contrast, the change in hypercapnic CO_2 reactivity following prazosin (-0.23 cm·s^{-1}·mmHg^{-1}) was similar to placebo (-0.31 cm·s^{-1}·mmHg^{-1}; $P = 0.50$ for interaction). These data indicate that the sympathetic nervous system contributes to CO_2 reactivity via α_1-adrenoreceptors; blocking this pathway with prazosin reduces CO_2 reactivity to hypocapnia but not hypercapnia.

cerebral blood flow; circulation; adrenergic

THE MAINTENANCE OF ADEQUATE cerebral blood flow (CBF) is achieved through a variety of physiological processes that buffer the cerebral circulation against changes in the physical and chemical environment (33). One such process is termed CO_2 reactivity, which refers to the vasoconstriction and vasodilatation of cerebral vessels in response to decreases and increases in the partial pressure of arterial CO_2 (Pa_{CO_2}; Ref. 1). Elevations in CBF with vasodilatation facilitates CO_2 washout from brain tissue during hypercapnia, whereas reductions in CBF with vasoconstriction during hypocapnia attenuate reductions in brain tissue CO_2. Thus CO_2 reactivity plays an important role in CBF and central pH control.

The precise mechanisms underpinning this vascular response are poorly understood. One area of uncertainty is the extent Pa_{CO_2}-evoked alterations in the cerebral vascular resistance are mediated through changes in cerebral sympathetic activity (21, 25, 28, 35, 41). This uncertainty has arisen partly because of interstudy differences in participant populations and experimental methods. For example, clinical studies (28, 41) have generally failed to demonstrate any sympathetic influence on CO_2 reactivity, whereas studies (21, 25, 35, 48) conducted on healthy subjects have produced mixed results. However, comparisons between healthy and diseased populations are liable to confounding because CO_2 reactivity is impaired in many diseases affecting the circulatory system (28, 41), including hypertension and stroke (36). Differences in the specificity of methods to modulate sympathetic activity may also contribute to the mixed results in healthy participants. The elimination of sympathetic activity through ganglionic blockade (trimethaphan), for example, has been shown to augment (21) or reduce (35) CO_2 reactivity. It should be noted that trimethaphan also affects cholinergic and histaminergic transmission (12); thus treatment effects cannot be ascribed purely to the elimination of sympathetic activity. Studies employing lower body negative pressure as a means of stimulating the sympathetic system have reported reductions (48) or no change (25) in CO_2 reactivity. These studies are limited by the fact that direct cerebral sympathetic neural recordings have failed to confirm the assumption that cerebral sympathetic outflow increases with baroreflex unloading (5, 6). Finally, most investigations have not included control trials to account for potential confounding due to time of day changes in CO_2 reactivity (2).

Given the aforementioned limitations and considering that CO_2 reactivity has become widely recognized as a surrogate of cerebrovascular reserve (26), clarification of whether sympathetic activity contributes to CO_2 reactivity is clearly required.

Therefore, the aim of this study was to directly assess the contribution of the sympathetic nervous system to CO_2 reactivity using a selective α_1-adrenergic blocking agent (prazosin). Since sympathetic excitation evokes vasoconstriction in most vascular beds, and the cerebrovasculature is known to receive sympathetic innervation, we hypothesized that in healthy individuals α_1-adrenergic blockade would attenuate CO_2 reactivity to hypocapnia (vasoconstriction) but not hypercapnia (vasodilatation).

METHODS

Ethical approval. Procedures were approved by the New Zealand Central Regional Ethics Committee and conformed to the standards set by the Declaration of Helsinki.

Participants. Eighteen healthy participants with a mean age of 23 yr (range 21–26) and a mean body mass index of 22.8 ± 1.7 kg/m were randomized to placebo ($n = 9$, 6 female) and active treatment groups ($n = 9$, 5 female). All participants were screened for respiratory, cardiovascular, and cerebrovascular disease and gave informed written consent. Participants were nonsmokers and were not taking any respiratory or cardiovascular medications. Our required sample size was determined a priori based on previous studies showing a 17–44% change in CO_2 reactivity following sympathetic blockade

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(21, 35). Pilot trials indicated that the average baseline CO_2 reactivity were ~2.54 cm·s^{-1}·mmHg^{-1}. Therefore, it was estimated that nine participants would provide >80% power to detect a minimal change of 0.43 cm·s^{-1}·mmHg^{-1} (i.e., 17%) in CO_2 reactivity, conservatively assuming a standard deviation of differences of 0.3 and a two-tailed significance level of 0.05.

Measurements. CBF velocity was measured in the M1 segment of the left or right middle cerebral artery (MCA) using 2-MHz pulsed wave transcranial Doppler ultrasound (ST3 Digital Transcranial Doppler System; Spencer Technologies, Seattle, WA). Continuous blood pressure was measured via finger photoelectric plethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Heart rate (HR) was recorded from a three-lead electrocardiogram (ADInstruments, Colorado Springs, CO), and partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) was sampled from a nasal cannula and measured using a gas analyzer (model ML206; ADInstruments). Data were attained continuously at 1 kHz per channel via an analog-to-digital converter (PowerLab/16SP ML795; ADInstruments) and stored for offline analysis.

Experimental protocol. All experiments were conducted with participants lying supine for safety reasons, recognizing that orthostatic intolerance is a common side effect of α_1-adrenergic blockade. Studies took place in the morning at 0900, and participants had arrived at the laboratory following a light breakfast at ~0700 and having abstained from coffee, alcohol, and strenuous exercise for ≥12 h before starting the study. Once the participants acclimatized to the equipment and laboratory environment, 6 min of baseline resting data were recorded. Thereafter, participants breathed a CO_2 gas mixture (5% CO_2 with 21% O_2 and balanced N_2) for ~100 s followed by ~100 s of voluntary hyperventilation until $P_{ET}CO_2$ had decreased 4 mmHg or more relative to baseline (8). After the pretreatment CO_2 reactivity testing was completed, the active treatment group participants ingested 0.05 mg/kg of the competitive α_1-adrenergic blocker prazosin (with ~250 ml water) as previously described (31), while the placebo group ingested a placebo pill with water. This dose of prazosin has been shown to block ~80% of the pressor response to phenylephrine in healthy normotensive participants who were of similar age and body mass index to those in the present study (20, 31). Participants then repeated the protocol 120 min postingestion to coincide with the peak plasma prazosin concentration (19). Participants were free to move around within the laboratory environment during the 120-min postingestion period but did not eat or drink. Herein the two groups are referred to as the α_1-adrenergic blockade and placebo group.

Data analysis. From the continuous blood pressure and MCA blood velocity waveforms, we determined beat-to-beat mean arterial pressure (MAP) and mean MCA blood velocity ($MCA_{V\ mean}$). CO_2 reactivity was quantified as the linear relationship between breath-to-breath changes in $P_{ET}CO_2$ and the average beat-to-beat $MCA_{V\ mean}$ and MAP values within successive breaths after accounting for known physical and physiological latencies. First, to account for the gas sampling delay associated with physical components of the breathing circuit, the entire $P_{ET}CO_2$ trace was left shifted relative to the $MCA_{V\ mean}$ and MAP time series by 2.6 s. Next, the physiological latency of the CO_2 reactivity response was identified as the time interval corresponding to the maximum positive cross-correlation between the $P_{ET}CO_2$ and $MCA_{V\ mean}$ time series, which was then time shifted to incorporate the delay (Fig. 1). Cross-correlation analysis is an accepted approach for estimating the stimulus-response latencies within physiological systems (37) such as the arterial baroreflex (47) and cerebral autoregulation (7, 43). No delays were introduced to the relation between MAP and $MCA_{V\ mean}$.

To estimate the linear relation between $P_{ET}CO_2$ and $MCA_{V\ mean}$, we employed linear mixed effects modeling analysis with repeated measures. This approach is a modification of the technique proposed by Dumville et al. (11), who employed multiple linear regression to derive CO_2 reactivity estimates. In contrast to conventional least squares regression, mixed effects models explicitly account for the fact that repeated $P_{ET}CO_2$ and $MCA_{V\ mean}$ measurements made within subjects are correlated in nature and therefore violate the case independence assumption required for least squares regression (24). Furthermore, whereas conventional regression analysis requires *x-y* data to be reduced to summary measures before secondary analysis using techniques such as ANOVA, mixed effects models analyzes the data in one step without losing valuable information concerning data precision as indicated by the standard error of individual slope estimates (3). Thus parameter estimates are weighted for precision and are more robust. $MCA_{V\ mean}$ was entered into the model as the primary outcome variable, $P_{ET}CO_2$ as the predictor variable, and MAP as a covariate to control for potential confounding associated with CO_2-driven changes in MAP. Random effects terms for subject, $P_{ET}CO_2$, condition, and intercept were added as the inclusion of these terms maximized the model fit (Akaike information criterion). This analysis was conducted for integrated CO_2 reactivity, which represents the relationship between $P_{ET}CO_2$ and $MCA_{V\ mean}$ across the entire range of $P_{ET}CO_2$ values, and repeated separately for the hypercapnic and hypocapnic regions. The cerebrovascular conductance index (CVC_i) was calculated as the $MCA_{V\ mean}$ divided by the MAP.

Statistical analysis. Linear mixed effects models were implemented as described above. To investigate the overall effects of α_1-adrenergic blockade vs. placebo on CO_2 reactivity, we tested for a group × treatment × $P_{ET}CO_2$ interaction as well as all lower order interactions

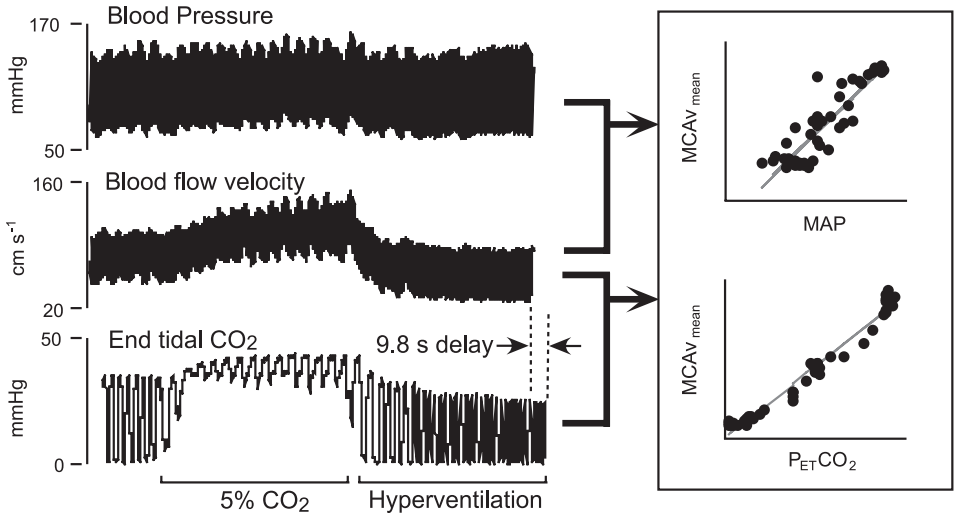

Fig. 1. Representative raw traces of blood pressure, middle cerebral artery (MCA) blood velocity, and partial pressure of nasal CO_2 during CO_2 reactivity testing. Progressive hypercapnia and hypocapnia was induced via 5% CO_2 inhalation and voluntary hyperventilation. The physiological latency of the CO_2 reactivity response was identified as the time interval corresponding to the maximum positive cross-correlation between the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) and mean MCA flow velocity ($MCA_{V\ mean}$) time series, which was then left shifted to incorporate the delay (9.8 s in this example trace). A strong association between $P_{ET}CO_2$ and $MCA_{V\ mean}$ was seen in all subjects. Strong associations were also seen between mean arterial pressure (MAP) and $MCA_{V\ mean}$, which justify our inclusion of MAP as a covariate in linear mixed effects model analysis (see METHODS).

Table 1. *Baseline parameters before and after treatment*

	α_1-Adrenergic Blockade		Placebo		*P* Value		
Baseline	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Treatment	Group	Interaction
HR, beats/min	63 ± 2.1	62 ± 2.1	62 ± 2.0	57 ± 2.0	<0.01	0.30	0.13
MAP, mmHg	70 ± 1.4	69 ± 1.9	74 ± 1.4	78 ± 3.6	0.49	<0.05	0.090
$MCA_{V\ mean}$, cm/s	68 ± 3.3	64 ± 3.0	66 ± 2.3	65 ± 2.1	0.11	0.93	0.25
$P_{ET}CO_2$, mmHg	37 ± 1.1	38 ± 1.2	38 ± 1.2	37 ± 1.1	0.81	0.85	0.10
CVC_i, $cm \cdot s^{-1} \cdot mmHg^{-1}$	0.97 ± 0.045	0.93 ± 0.037	0.89 ± 0.029	0.85 ± 0.044	0.093	0.11	0.78

Values are means ± SE for the 6-min baseline recordings. HR, heart rate; MAP, mean arterial pressure; MAP amplitude, mean arterial pressure fluctuation amplitude; $MCA_{V\ mean}$, mean middle cerebral artery blood flow velocity; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; CVC_i, cardiovascular conductance index.

and fixed effects. A significant three-way interaction indicates that the changes in the slope relating $P_{ET}CO_2$ and $MCA_{V\ mean}$ (i.e., CO_2 reactivity) differed between the active treatment and placebo control groups. Statistically significant three-way interactions were followed up with tests for a treatment × $P_{ET}CO_2$ interactions to determine whether CO_2 reactivity altered in response to prazosin and placebo. Within-subject (before vs. after treatment) and between-subject (α_1-adrenergic blockade vs. placebo) differences in baseline cardiovascular and respiratory parameters were also assessed using linear mixed effects models. Statistically significant two-way interactions were followed up with pair-wise contrasts. Assessment of a priori planned pair-wise comparisons for cardiovascular and respiratory parameters between hypercapnia and hypocapnia vs. baseline were done using Student's paired *t*-tests. *P* values were adjusted using the Holm-Bonferroni method to control for the inflation of type 1 error associated with multiple testing (18). All data were analyzed using custom-written software in LabView 11 (National Instruments, Austin, TX) and SPSS (IBM SPSS statistics version 19, Surrey, UK). For consistency, all data are expressed as means ± SE. Significance was established a priori at $P < 0.05$.

RESULTS

Baseline parameters. The effects of α_1-adrenergic blockade and placebo intervention on baseline parameters are shown in Table 1. HR was lower following treatment ($P < 0.01$) and comparable between groups ($P = 0.30$ for group effect, $P = 0.13$ for interaction). There were no interaction or treatment main effects for MAP, indicating that treatment responses to α_1-adrenergic blockade and placebo were similar. Interaction and main effects were not significant for $MCA_{V\ mean}$, $P_{ET}CO_2$, and CVC_i, indicating that neither α_1-adrenergic blockade nor placebo affected these parameters at baseline.

CO_2 reactivity. A representative example of blood pressure, MCAv, and expired CO_2 changes during CO_2 reactivity testing for one subject is shown in Fig. 1. The cardiovascular and respiratory responses to 5% CO_2 inhalation and voluntary hyperventilation are summarized in Table 2. Inhalation of 5% CO_2 and subsequent hyperventilation resulted in marked increases and decreases in $P_{ET}CO_2$, respectively (Table 2). The resultant hypercapnia and hypocapnia consistently altered $MCA_{V\ mean}$ and CVC_i and inconsistently altered MAP or HR (Table 2). The magnitude of hypercapnia was similar between the α_1-adrenergic blockade and placebo control groups both before and after treatment (main effect for group, $P = 0.98$; main effect for treatment, $P = 0.43$; interaction, $P = 0.17$). Likewise, the magnitude of hypocapnia was similar between the groups and study conditions (main effect for group, $P = 0.65$; main effect for treatment, $P = 0.41$; interaction, $P = 0.72$). Thus the ranges of hypercapnia and hypocapnia achieved before and after α_1-adrenergic blockade were similar to each other (before vs. after) and similar to placebo.

Linear mixed-effects analysis showed that typically $P_{ET}CO_2$ and MAP were both significant predictors of $MCA_{V\ mean}$ dynamics, justifying the inclusion of MAP as a covariate. The effects of α_1-adrenergic blockade or placebo on integrated, hypercapnic, and hypocapnic CO_2 reactivity are summarized in Table 3 and in Fig. 2, which highlights the magnitude of the treatment effects. Integrated, hypocapnic, and hypercapnic CO_2 reactivity in both the α_1-adrenergic blockade and placebo groups were reduced following treatment (Fig. 2). However, three-way interaction (group × treatment × CO_2) effects were observed only for the integrated and hypocapnic response, indicating

Table 2. *Baseline cardiovascular and respiratory parameters before and after treatment*

	α_1-Adrenergic Blockade			Placebo		
	Baseline	5% CO_2	Hyperventilation	Baseline	5% CO_2	Hyperventilation
Pretreatment						
$P_{ET}CO_2$, mmHg	37 ± 2.8	45 ± 3.1†	29 ± 2.8†	37 ± 1.0	46 ± 1.1†	30 ± 1.2†
$MCA_{V\ mean}$, cm/s	68 ± 9.8	87 ± 9.8†	49 ± 7.5†	67 ± 6.9	93 ± 11†	47 ± 4.6†
HR, beats/min	59 ± 2.2	61 ± 2.8	64 ± 3.0*	61 ± 2.1	63 ± 2.7	68 ± 3.6
CVCi, $cm \cdot s^{-1} \cdot mmHg^{-1}$	0.86 ± 0.14	1.1 ± 0.16†	0.66 ± 0.12†	0.78 ± 0.10	1.0 ± 0.10†	0.67 ± 0.085†
MAP, mmHg	80 ± 12	81 ± 10	76 ± 11	83 ± 9.6	85 ± 11	78 ± 6.5
Posttreatment						
$P_{ET}CO_2$, mmHg	38 ± 4.8	45 ± 5.3†	29 ± 3.6†	36 ± 1.0	45 ± 1.1†	28 ± 1.2†
$MCAv_{mean}$, cm/s	63 ± 6.7	83 ± 13†	49 ± 9.1†	65 ± 10	88 ± 9.8†	54 ± 9.8†
HR, beats/min	62 ± 2.4	65 ± 2.6*	66 ± 3.3	59 ± 3.0	59 ± 2.0	65 ± 3.4*
CVCi, $cm \cdot s^{-1} \cdot mmHg^{-1}$	0.91 ± 0.17	1.2 ± 0.22†	0.73 ± 0.17†	0.78 ± 0.10	1.0 ± 0.10†	0.67 ± 0.089†
MAP, mmHg	71 ± 7.0	72 ± 5.0	67 ± 5.0	84 ± 7.3	86 ± 7.2*	81 ± 7.8†

Values are means ± SE. Baseline refers to the 6 s immediately preceding 5% CO_2 exposure. Hypercapnia and hypocapnia refers to the maximum and minimum $P_{ET}CO_2$, during the CO_2 reactivity test. *$P < 0.05$ vs. baseline; †$P < 0.01$ vs. baseline.

Table 3. *Effects of α_1-adrenergic blockade and placebo on CO_2 reactivity*

CO_2 Reactivity, cm s^{-1} $mmHg^{-1}$	α_1-Adrenergic Blockade		Placebo	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Integrated response	2.5 ± 0.14	2.1 ± 0.14†	2.7 ± 0.14	2.5 ± 0.14†
Hypocapnic response	1.9 ± 0.17	1.4 ± 0.16†	2.4 ± 0.16	2.2 ± 0.17*
Hypercapnic response	2.7 ± 0.15	2.5 ± 0.15*	3.0 ± 0.15	2.7 ± 0.15†

Values are means ± SE. *$P < 0.05$ vs. pretreatment. †$P < 0.01$ vs. pretreatment.

that, after accounting for time-controlled changes (placebo group), α_1-adrenergic blockade blunted integrated and hypocapnic CO_2 reactivity but not the CO_2 reactivity response to hypercapnia (Fig. 2). No significant main effects or interactions were found for the integrated, hypercapnic, or hypocapnic CO_2 reactivity delays (Table 4), indicating that α_1-adrenergic blockade did not alter CO_2 reactivity latency.

DISCUSSION

The main findings of this study were that α_1-adrenergic blockade: *1*) blunted the decrease in CBF evoked by hypocapnia but not the increase in CBF evoked by hypercapnia; and *2*) did not alter the CO_2 reactivity latency to hypocapnia or hypercapnia. These results indicate that the sympathetic system contributes to the cerebral vasoconstrictor response to hypocapnia rather than hypercapnia and that the putative influence of α_1-adrenergic activity on CO_2 reactivity is limited to the magnitude and not the latency of the response. Furthermore, hypercapnic and hypocapnic CO_2 reactivity was blunted in placebo controls, indicative of time-influenced changes in cerebrovascular responsiveness. In the absence of time controls, we would have misleadingly concluded that sympathetic activity modulates CO_2 reactivity to hypercapnia. Therefore, our findings highlight the importance of placebo controls, which have been lacking in most, if not all, previous investigations on the sympathetic regulation of CO_2 reactivity.

Sympathetic regulation of the cerebrovasculature. Although the relevance of the sympathetic system in human CBF control has been the subject of intense debate (44, 46), our observation that α_1-adrenergic receptor blockade blunted CO_2 reactivity to hypocapnia suggests that the cerebral vasoconstriction is partly mediated by sympathetic activity. This proposition is physiologically plausible given that the cerebrovasculature purportedly receives rich sympathetic innervation in many animal species (39), and α_1-adrenergic receptor stimulation is known to evoke vascular smooth muscle constriction in most vascular beds (16). Moreover, norepinephrine plasma kinetic measurements made with internal jugular venous sampling have been shown to reflect cerebrovascular sympathetic activity from outside the blood brain barrier (30), and recent studies have documented impaired cerebral pressure-flow autoregulation following α_1-adrenergic receptor blockade (15, 31), which is indicative of active cerebral sympathetic control (5). Our results support these prior observations and extend them by showing that cerebral sympathetic activity contributes to CBF regulation against dynamic fluctuations in arterial CO_2.

The notion that hypocapnia might trigger cerebral sympathetic excitation has important implications. Previous studies have shown that muscle (42) and cardiac sympathetic activities (10) increase in response to hypercapnia, not hypocapnia. Therefore, assuming that CO_2-driven changes in regional sympathetic outflow are all mediated through common afferent pathways, the elevation of cerebral sympathetic activity with hypocapnia implies that sympathetic outflow to the brain might be differentially regulated from the outflow to other vascular beds. Although we did not perform regional sympathetic recordings to verify this possibility, it has been shown that cerebral sympathetic activity in lambs "paradoxically" increases with transient hypertension but not with hypotension (6). This pattern of activity differs from that associated with regulation of systemic vascular resistance and arterial blood pressure (38). Thus it is possible that CO_2 may activate superior cervical ganglion neurons in a pattern that does not simply parallel the outflow to other (e.g., muscle) vascular beds. Speculatively, such differential regulation to both baroreflex and chemoreflex stimuli may be teleologically advantageous under situations where CBF stabilization is paramount. For example, cerebral sympathetic excitation in response to hypertension may be an adaptive mechanism that protects the cerebral circulation against excess cerebral perfusion (5). Likewise, cerebral sympathetic excitation during hypocapnia might facilitate vasoconstriction and central pH restoration by reducing CBF and therefore the washout of brain CO_2. If confirmed, our findings may help explain why sympathetic dysfunction is associated with adverse cerebrovascular outcomes.

While our findings implicate the sympathetic system in CO_2 reactivity to hypocapnia, we recognize that the sympathetic blockade, which has been shown to block ~80% of the peripheral vasoconstriction response (20, 32), only reduced CO_2 reactivity to hypocapnia by ~26%. Therefore, hypocapnia-induced vasoconstriction appears to be largely driven independently of α_1-adrenergic receptor stimulation within the ranges of $P_{ET}CO_2$ we studied. In this context, potential mechanisms for the residual cerebral hypocapnic reactivity warrant brief consideration. The most likely mechanism is that hypocapnia-induced cerebral vasoconstriction is initiated by an increase in local pH. For example, an increase in pH in the vascular smooth muscle decreases the open-state probability of pH sensitive K^+ channels (e.g., K_{ATP}) leading to depolarization of the cell membrane, an increase in cytosolic Ca^{2+}, and reduction in vessel caliber (29). This notion is based on studies implicating the reciprocal response during hypercapnic vasodilatation (13, 22). Another possibility is that alterations in vasoactive factors play a role in hypocapnic cerebral vasocon-

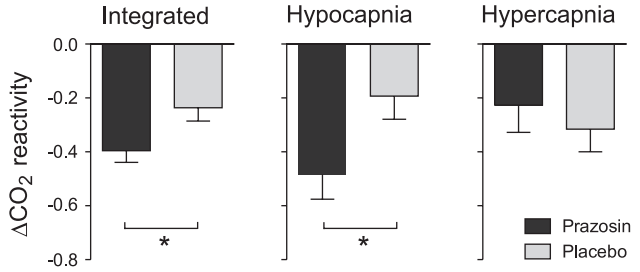

Fig. 2. Effect of α_1-adrenergic blockade or placebo on integrated, hypocapnia, and hypercapnia CO_2 reactivity ($cm \cdot s^{-1} \cdot mmHg^{-1}$). Bars show the change scores following treatment. *$P < 0.05$ prazosin vs. placebo. For hypercapnia, $P = 0.50$.

Table 4. *Effects of α_1-adrenergic blockade and placebo on CO_2 reactivity latency*

	α_1-Adrenergic Blockade		Placebo		*P* Value		
Latency, s	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Treatment	Group	Interaction
Integrated	13 ± 2.3	11 ± 3.0	13 ± 2.8	13 ± 3.4	0.13	0.59	0.19
Hypocapnic	12 ± 1.9	9.1 ± 2.6	10 ± 3.0	11 ± 5.0	0.15	0.78	0.10
Hypercapnic	14 ± 2.1	12 ± 4.0	14 ± 2.7	14 ± 1.7	0.31	0.25	0.21

Values are means ± SE in seconds.

striction, although the precise mechanisms remain unclear. Peebles et al. (34) cannulated the radial artery and internal jugular vein to directly examine the role of vasoactive factors during air breathing and alterations in Pa_{CO_2} in healthy humans. They found similar levels of endothelin-1, NO metabolites, and adrenomedullin during air breathing and graded hypocapnia down to 24 mmHg $P_{ET}CO_2$, which is beneath that in the present study. The identification of vasoactive factors responsible for hypocapnic cerebrovascular reactivity extends beyond the scope of our investigation but clearly warrants further research.

In contrast to hypocapnia, α_1-adrenergic blockade did not blunt the CO_2 reactivity to hypercapnia beyond any time-controlled changes (placebo group). One interpretation is that sympathetic activity does not play an obligatory role in modulating the vasodilatation response to hypercapnia, which is conceivable given that α_1-adrenergic stimulation causes vascular smooth muscle constriction rather than dilatation. Our results do not negate the potential for sympathetic activity to effect vasodilatation via β-adrenergic receptor stimulation, although previous studies (14, 23, 45) have consistently failed to demonstrate an effect of β-adrenergic blockade or stimulation on CBF. Interestingly, the placebo group had blunted integrated, hypercapnic, and hypocapnic CO_2 reactivity indicative of time-influenced changes in cerebrovascular responses to CO_2. Given our study was designed specifically to examine sympathetic influence on CO_2 reactivity, we cannot explicate the mechanisms underpinning the unexpected changes observed in the placebo group (speculatively, influences could include alterations in intrinsic vasoactive factors such as nitric oxide or endothelin-1). Nevertheless, our findings do highlight the need for physiological studies to incorporate placebo conditions. In the absence of time-controlled trials, we would have overestimated the blunting of CO_2 reactivity to hypocapnia and falsely concluded blunting to hypercapnia following α_1-adrenergic blockade.

Comparison to previous studies. Several contrasts between this study and previous investigations into the role of the sympathetic system in CO_2 reactivity warrant discussion. One important feature is that all participants in this study were healthy without any preexisting medical history. This may explain why our findings differed from prior investigations conducted in patients afflicted with neurological conditions including recent cerebral ischemia and stroke (28, 41). Furthermore, this study examined $MCA_{V\ mean}$ responses to dynamic breath-to-breath changes in $P_{ET}CO_2$ rather than the cerebrovascular response to steady-state changes in $P_{ET}CO_2$ (21, 25). Steady-state CO_2 reactivity reflects the net effect of all mechanisms engaged by changes in Pa_{CO_2} and therefore does not take into account the time in which vascular responses occur. Speculatively, the sympathetic input to CO_2 reactivity may be more difficult to detect under steady-state conditions due to functional redundancies between different contributing mechanisms. This methodological difference may partly explain why previous studies employing steady-state approaches have failed to identify a sympathetic influence on CO_2 reactivity (25). We found that the average CO_2 reactivity delay was ~12 s, which is consistent with recent work by Hamner et al. (15) showing that human cerebral sympathetic control operates with a ~0.08 Hz (i.e., 12.5 s) dynamic time constant. This delay did not change with α_1-adrenergic receptor blockade, indicating that the putative influence of α_1-adrenergic activity on CO_2 reactivity is limited to the magnitude and not the latency of the response.

Methodological considerations and limitations. The results of this study need to be interpreted in cognizance of several methodological considerations. First, blood flow velocity measurements reflect changes in volumetric blood flow only if the diameter of the MCA remain constant. Previous studies employing the same CO_2 reactivity test protocol have confirmed that MCA diameter does remain constant during a range of physiological perturbations including mild to moderate hypocapnia and hypercapnia (40). Therefore, we consider it reasonable to assume that changes in MCAv measured via transcranial Doppler ultrasound were proportional to changes in CBF. Second, it has previously been suggested that CO_2-mediated changes in blood pressure may confound CO_2 reactivity estimation (11, 17). To account for this potential confounding factor, MAP was included as a covariate to explicitly control for its effects when estimating the coefficients relating $P_{ET}CO_2$ to $MCA_{V\ mean}$. Our approach is therefore similar to the method proposed by Dumville et al. (11), who employed a multiple regression model and showed that MAP was a significant predictor of $MCA_{V\ mean}$ dynamics (in 96% of their participants) and that MAP-adjusted CO_2 reactivity was 20% lower compared with the conventional ratio between relative $MCA_{V\ mean}$ and $P_{ET}CO_2$. Nevertheless, it needs to be acknowledged that neither multiple regression nor linear mixed models explicitly account for nonlinearities in the dynamic pressure-flow relationship of the cerebral circulation. The development of methods that do account for such nonlinearities may further enhance the estimation of CO_2 reactivity. Third, CO_2 reactivity impairment is associated with an increased risk of stroke (27) and subarachnoid hemorrhage (9), and predicts poorer prognosis in traumatic brain injury (4). Further studies are needed to confirm whether cerebral sympathetic dysfunction underpins the deficits in CO_2 reactivity seen in these cerebrovascular conditions. Finally, additional research is needed to verify our speculation that CO_2-evoked changes in efferent cerebral sympathetic outflow is differentially regulated from efferent sympathetic outflow to the peripheral (e.g., muscle) vasculature. To our knowledge, such concurrent recordings have not been performed in humans during dynamic changes in $P_{ET}CO_2$.

Although it may not be practicable to obtain cerebral sympathetic nerve recordings in conscious human volunteers, a viable alternative is to quantify transcranial plasma norepinephrine spillover from internal jugular venous blood samples taken before and during a hypocapnic challenge (30).

Conclusion. This study indicates that the sympathetic nervous system contributes to CO_2 reactivity via α_1-adrenoreceptors as blocking this pathway with prazosin reduced CO_2 reactivity to hypocapnia but not hypercapnia. This observation implicates sympathetic involvement in human CBF regulation specifically against hypocapnia. As different conclusions would have been drawn in the absence of placebo trials, our findings also highlight the importance of time controls, which have been lacking in previous investigations on the sympathetic regulation of CO_2 reactivity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.-C.T. conception and design of research; O.G.B., B.A.M., H.M.H., and Y.-C.T. performed experiments; O.G.B., B.A.M., and Y.-C.T. analyzed data; K.C.P., O.G.B., B.A.M., H.M.H., and Y.-C.T. interpreted results of experiments; Y.-C.T. prepared figures; K.C.P., O.G.B., and Y.-C.T. drafted manuscript; K.C.P., O.G.B., B.A.M., H.M.H., and Y.-C.T. edited and revised manuscript; K.C.P., O.G.B., B.A.M., H.M.H., and Y.-C.T. approved final version of manuscript.

REFERENCES

1. **Ainslie PN, Duffin J.** Integration of cerebrovascular CO_2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol* 296: R1473–R1495, 2009.
2. **Ameriso SF, Mohler JG, Suarez M, Fisher M.** Morning reduction of cerebral vasomotor reactivity. *Neurology* 44: 1907–1909, 1994.
3. **Atkinson G, Batterham AM, Jones H, Taylor CE, Willie CK, Tzeng YC.** Appropirate within-subjects statistical models for the analysis of baroreflex sensitivity. *Clin Physiol Funct Imaging* 31: 80–82, 2011.
4. **Bouma GJ, Muizelaar JP.** Cerebral blood flow, cerebral blood volume, and cerebrovascular reactivity after severe head injury. *J Neurotrauma* 9, *Suppl 1*: S333–S348, 1992.
5. **Cassaglia PA, Griffiths RI, Walker AM.** Cerebral sympathetic nerve activity has a major regulatory role in the cerebral circulation in REM sleep. *J Appl Physiol* 106: 1050–1056, 2009.
6. **Cassaglia PA, Griffiths RI, Walker AM.** Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure. *Am J Physiol Regul Integr Comp Physiol* 294: R1255–R1261, 2008.
7. **Chiu CC, Yeh SJ.** Assessment of cerebral autoregulation using time-domain cross-correlation analysis. *Comput Biol Med* 31: 471–480, 2001.
8. **Deegan BM, Sorond FA, Galica A, Lipsitz LA, O'Laighin G, Serrador JM.** Elderly women regulate brain blood flow better than men do. *Stroke* 42: 1988–1993, 2011.
9. **Dernbach PD, Little JR, Jones SC, Ebrahim ZY.** Altered cerebral autoregulation and CO2 reactivity after aneurysmal subarachnoid hemorrhage. *Neurosurgery* 22: 822–826, 1988.
10. **Downing SE, Siegel JH.** Baroreceptor and chemoreceptor influences on sympathetic discharge to the heart. *Am J Physiol* 204: 471–479, 1963.
11. **Dumville J, Panerai RB, Lennard NS, Naylor AR, Evans DH.** Can cerebrovascular reactivity be assessed without measuring blood pressure in patients with carotid artery disease? *Stroke* 29: 968–974, 1998.
12. **Fahmy NR, Soter NA.** Effects of trimethaphan on arterial blood histamine and systemic hemodynamics in humans. *Anesthesiology* 62: 562–566, 1985.
13. **Faraci FM, Breese KR, Heistad DD.** Cerebral vasodilation during hypercapnia. Role of glibenclamide-sensitive potassium channels and nitric oxide. *Stroke* 25: 1679–1683, 1994.
14. **Griffith DN, James IM, Newbury PA, Woollard ML.** The effect of beta-adrenergic receptor blocking drugs on cerebral blood flow. *Br J Clin Pharmacol* 7: 491–494, 1979.
15. **Hamner JW, Tan CO, Lee K, Cohen MA, Taylor JA.** Sympathetic control of the cerebral vasculature in humans. *Stroke* 41: 102–109, 2010.
16. **Henriksen O.** Sympathetic reflex control of blood flow in human peripheral tissues. *Acta Physiol Scand Suppl* 603: 33–39, 1991.
17. **Hetzel A, Braune S, Guschlbauer B, Dohms K.** CO2 reactivity testing without blood pressure monitoring? *Stroke* 30: 398–401, 1999.
18. **Holm S.** A simple sequentially rejective multiple test procedure. *Scand J Clin Statist* 65–70, 1979.
19. **Jaillon P.** Clinical pharmacokinetics of prazosin. *Clin Pharmacokinet* 5: 365–376, 1980.
20. **Jones H, Lewis NC, Green DJ, Ainslie PN, Lucas SJ, Tzeng YC, Grant EJ, Atkinson G.** α_1-Adrenoreceptor activity does not explain lower morning endothelial-dependent, flow-mediated dilation in humans. *Am J Physiol Regul Integr Comp Physiol* 300: R1437–R1442, 2011.
21. **Jordan J, Shannon JR, Diedrich A, Black B, Costa F, Robertson D, Biaggioni I.** Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension* 36: 383–388, 2000.
22. **Kinoshita H, Katusic ZS.** Role of potassium channels in relaxations of isolated canine basilar arteries to acidosis. *Stroke* 28: 433–437; discussion 437–438, 1997.
23. **Kuriyama Y, Nakamura M, Kyougoku I, Sawada T.** Effects of carvedilol on cerebral blood flow and its autoregulation in previous stroke patients with hypertension. *Eur J Clin Pharmacol* 38, *Suppl 2*: S120–121, 1990.
24. **Lazic SE.** The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC Neurosci* 11: 5, 2010.
25. **LeMarbre G, Stauber S, Khayat RN, Puleo DS, Skatrud JB, Morgan BJ.** Baroreflex-induced sympathetic activation does not alter cerebrovascular CO2 responsiveness in humans. *J Physiol* 551: 609–616, 2003.
26. **Maeda H, Matsumoto M, Handa N, Hougaku H, Ogawa S, Itoh T, Tsukamoto Y, Kamada T.** Reactivity of cerebral blood flow to carbon dioxide in various types of ischemic cerebrovascular disease: evaluation by the transcranial Doppler method. *Stroke* 24: 670–675, 1993.
27. **Markus H, Cullinane M.** Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain* 124: 457–467, 2001.
28. **Meyer JS, Shimazu K, Okamoto S, Koto A, Ouchi T, Sari A, Ericsson AD.** Effects of alpha adrenergic blockade on autoregulation and chemical vasomotor control of CBF in stroke. *Stroke* 4: 187–200, 1973.
29. **Mirro R, Lowery-Smith L, Armstead WM, Shibata M, Zuckerman SL, Leffler CW.** Cerebral vasoconstriction in response to hypocapnia is maintained after ischemia/reperfusion injury in newborn pigs. *Stroke* 23: 1613–1616, 1992.
30. **Mitchell DA, Lambert G, Secher NH, Raven PB, van Lieshout J, Esler MD.** Jugular venous overflow of noradrenaline from the brain: a neurochemical indicator of cerebrovascular sympathetic nerve activity in humans. *J Physiol* 587: 2589–2597, 2009.
31. **Ogoh S, Brothers RM, Eubank WL, Raven PB.** Autonomic neural control of the cerebral vasculature: acute hypotension. *Stroke* 39: 1979–1987, 2008.
32. **Ogoh S, Hayashi N, Inagaki M, Ainslie PN, Miyamoto T.** Interaction between the ventilatory and cerebrovascular responses to hypo- and hypercapnia at rest and during exercise. *J Physiol* 586: 4327–4338, 2008.
33. **Paulson OB, Waldemar G, Schmidt JF, Strandgaard S.** Cerebral circulation under normal and pathologic conditions. *Am J Cardiol* 63: 2C–5C, 1989.
34. **Peebles KC, Richards AM, Celi L, McGrattan K, Murrell CJ, Ainslie PN.** Human cerebral arteriovenous vasoactive exchange during alterations in arterial blood gases. *J Appl Physiol* 105: 1060–1068, 2008.
35. **Przybylowski T, Bangash MF, Reichmuth K, Morgan BJ, Skatrud JB, Dempsey JA.** Mechanisms of the cerebrovascular response to apnoea in humans. *J Physiol* 548: 323–332, 2003.
36. **Reinhard M, Roth M, Muller T, Czosnyka M, Timmer J, Hetzel A.** Cerebral autoregulation in carotid artery occlusive disease assessed from

spontaneous blood pressure fluctuations by the correlation coefficient index. *Stroke* 34: 2138–2144, 2003.
37. **Rowley AB, Payne SJ, Tachtsidis I, Ebden MJ, Whiteley JP, Gavaghan DJ, Tarassenko L, Smith M, Elwell CE, Delpy DT.** Synchronization between arterial blood pressure and cerebral oxyhaemoglobin concentration investigated by wavelet cross-correlation. *Physiol Meas* 28: 161–173, 2007.
38. **Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, Eckberg DL.** Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol Heart Circ Physiol* 276: H1691–H1698, 1999.
39. **Sandor P.** Nervous control of the cerebrovascular system: doubts and facts. *Neurochem Int* 35: 237–259, 1999.
40. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL.** MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31: 1672–1678, 2000.
41. **Skinhoj E.** The sympathetic nervous system and the regulation of cerebral blood flow in man. *Stroke* 3: 711–716, 1972.
42. **Somers VK, Mark AL, Zavala DC, Abboud FM.** Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *J Appl Physiol* 67: 2101–2106, 1989.
43. **Steinmeier R, Bauhuf C, Hubner U, Hofmann RP, Fahlbusch R.** Continuous cerebral autoregulation monitoring by cross-correlation analysis: evaluation in healthy volunteers. *Crit Care Med* 30: 1969–1975, 2002.
44. **Strandgaard S, Sigurdsson ST.** Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Counterpoint: Sympathetic nerve activity does not influence cerebral blood flow. *J Appl Physiol* 105: 1366–1367; discussion 1367–1368, 2008.
45. **Testa R, Rodriguez G, Dagnino F, Grasso A, Gris A, Marenco S, Nobili F, Risso D, Rosadini G, Celle G.** Effects of beta-adrenoreceptor antagonists on cerebral blood flow of cirrhotic patients with portal hypertension. *J Clin Pharmacol* 31: 136–139, 1991.
46. **van Lieshout JJ, Secher NH.** Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Point: Sympathetic activity does influence cerebral blood flow. *J Appl Physiol* 105: 1364–1366, 2008.
47. **Westerhof BE, Gisolf J, Stok WJ, Wesseling KH, Karemaker JM.** Time-domain cross-correlation baroreflex sensitivity: performance on the EUROBAVAR data set. *J Hypertens* 22: 1371–1380, 2004.
48. **Zhang P, Huang G, Shi X.** Cerebral vasoreactivity during hypercapnia is reset by augmented sympathetic influence. *J Appl Physiol* 110: 352–358, 2011.