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 CO₂, there is no consensus on whether the sympathetic nervous system plays a role in regulating cerebrovascular responses to changes in arterial CO₂. To address this question, we investigated human cerebrovascular CO₂ reactivity in healthy participants randomly assigned to the α₁-adrenoceptor 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 (MCAV mean), and partial pressure of end-tidal CO₂ (PETCO₂) during 5% CO₂ inhalation and voluntary hyperventilation. CO₂ reactivity was quantified as the slope of the linear relationship between breath-to-breath PETCO₂ and the average MCAVmean within successive breathes after accounting for MAP as a covariate. Prazosin did not alter resting HR, PETCO₂, MAP, or MCAV mean. The reduction in hypocapnic CO₂ 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 CO₂ 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 CO₂ reactivity via α₁-adrenoceptors; blocking this pathway with prazosin reduces CO₂ reactivity to hypocapnia but not hypercapnia.

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 CO₂ reactivity, which refers to the vasodilatation and vasodilation of cerebral vessels in response to decreases and increases in the partial pressure of arterial CO₂ (PaCO₂; Ref. 1). Elevations in CBF with vasodilatation facilitate CO₂ washout from brain tissue during hypocapnia, whereas reductions in CBF with vasoconstriction during hypercapnia attenuate reductions in brain tissue CO₂. Thus CO₂ 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 PaCO₂-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 CO₂ 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 CO₂ 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) CO₂ 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 CO₂ reactivity. These studies are limited by the fact that direct central 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 CO₂ reactivity (2).

Given the aforementioned limitations and considering that CO₂ reactivity has become widely recognized as a surrogate of cerebrovascular reserve (26), clarification of whether sympathetic activity contributes to CO₂ reactivity is clearly required.

Therefore, the aim of this study was to directly assess the contribution of the sympathetic nervous system to CO₂ reactivity using a selective α₁-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 α₁-adrenergic blockade would attenuate CO₂ 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/m² 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 CO₂ reactivity following sympathetic blockade...
Pilot trials indicated that the average baseline CO2 reactivity was \( \sim 2.54 \, \text{cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1} \). Therefore, it was estimated that nine participants would provide >80% power to detect a minimal change of 0.43 cm \( \cdot \) s \(^{-1} \cdot \) mmHg \(^{-1} \) (i.e., 17%) in CO2 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 photoplethysmography (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 CO2 (\( \text{PETCO}_2 \)) 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 \( \alpha_1 \)-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 \( \geq 12 \) 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 CO2 gas mixture (5% CO2 with 21% O2 and balanced N2) for \( \sim 100 \) s followed by \( \sim 100 \) s of voluntary hyperventilation until \( \text{PETCO}_2 \) had decreased 4 mmHg or more relative to baseline (8). After the pretreatment CO2 reactivity testing was completed, the active treatment group participants ingested 0.05 mg/kg of the competitive \( \alpha_1 \)-adrenergic blocker prazosin (with \( \sim 250 \) 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 \( \sim 80\% \) 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 postgestion to coincide with the peak plasma prazosin concentration (19). Participants were free to move around within the laboratory environment during the 120-min postgestion period but did not eat or drink. Herein the two groups are referred to as the \( \alpha_1 \)-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 (MCAV\( \text{mean} \)). CO2 reactivity was quantified as the linear relationship between breath-to-breath changes in \( \text{PETCO}_2 \) and the average beat-to-beat MCAV\( \text{mean} \) 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 \( \text{PETCO}_2 \) trace was left shifted relative to the MCAV\( \text{mean} \) and MAP time series by 2.6 s. Next, the physiological latency of the CO2 reactivity response was identified as the time interval corresponding to the maximum positive cross-correlation between the \( \text{PETCO}_2 \) and MCAV\( \text{mean} \) 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 MCAV\( \text{mean} \).

To estimate the linear relation between \( \text{PETCO}_2 \) and MCAV\( \text{mean} \), 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 CO2 reactivity estimates. In contrast to conventional least squares regression, mixed effects models explicitly account for the fact that repeated \( \text{PETCO}_2 \) and MCAV\( \text{mean} \) 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. MCAV\( \text{mean} \) was entered into the model as the primary outcome variable, \( \text{PETCO}_2 \) as the predictor variable, and MAP as a covariate to control for potential confounding associated with CO2-driven changes in MAP. Random effects terms for subject, \( \text{PETCO}_2 \) condition, and intercept were added as the inclusion of these terms maximized the model fit (Akaike information criterion). This analysis was conducted for integrated CO2 reactivity, which represents the relationship between \( \text{PETCO}_2 \) and MCAV\( \text{mean} \) across the entire range of \( \text{PETCO}_2 \) values, and repeated separately for the hypercapnic and hypocapnic regions. The cerebrovascular conductance index (CVCi) was calculated as the MCAV\( \text{mean} \) divided by the MAP.

**Statistical analysis.** Linear mixed effects models were implemented as described above. To investigate the overall effects of \( \alpha_1 \)-adrenergic blockade vs. placebo on CO2 reactivity, we tested for a group \( \times \) treatment \( \times \) \( \text{PETCO}_2 \) interaction as well as all lower order interactions.
and fixed effects. A significant three-way interaction indicates that the changes in the slope relating \( \text{PETCO}_2 \) and MCAV mean (i.e., CO2 reactivity) differed between the active treatment and placebo control groups. Statistically significant three-way interactions were followed up with tests for a treatment \( \times \text{PETCO}_2 \) interactions to determine whether CO2 reactivity altered in response to prazosin and placebo. Within-subject (before vs. after treatment) and between-subject (\( \alpha_1 \)-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 hypcapnia 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 I 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 \( \pm \) SE. Significance was established a priori at \( P < 0.05 \).

RESULTS

Baseline parameters. The effects of \( \alpha_1 \)-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 \( \alpha_1 \)-adrenergic blockade and placebo were similar. Interaction and main effects were not significant for MCAV mean, \( \text{PETCO}_2 \), and CVCi, indicating that neither \( \alpha_1 \)-adrenergic blockade nor placebo affected these parameters at baseline.

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

<table>
<thead>
<tr>
<th></th>
<th>( \alpha_1 )-Adrenergic Blockade</th>
<th>Placebo</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>63 ( \pm ) 2.1</td>
<td>62 ( \pm ) 2.1</td>
<td>62 ( \pm ) 2.0</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>70 ( \pm ) 1.4</td>
<td>69 ( \pm ) 1.9</td>
<td>74 ( \pm ) 1.4</td>
</tr>
<tr>
<td>MCAV mean, cm/s</td>
<td>68 ( \pm ) 3.8</td>
<td>64 ( \pm ) 3.0</td>
<td>66 ( \pm ) 3.0</td>
</tr>
<tr>
<td>( \text{PETCO}_2 ), mmHg</td>
<td>37 ( \pm ) 1.1</td>
<td>38 ( \pm ) 1.2</td>
<td>38 ( \pm ) 1.2</td>
</tr>
<tr>
<td>CVCi, cm ( \cdot ) s ( ^{-1} ) mmHg ( ^{-1} )</td>
<td>0.97 ( \pm ) 0.045</td>
<td>0.93 ( \pm ) 0.037</td>
<td>0.89 ( \pm ) 0.029</td>
</tr>
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</table>

Values are means \( \pm \) SE for the 6-min baseline recordings. HR, heart rate; MAP, mean arterial pressure; MAP amplitude, mean arterial pressure fluctuation amplitude; MCAV mean, mean middle cerebral artery blood flow velocity; \( \text{PETCO}_2 \), partial pressure of end-tidal CO2; CVCi, cardiovascular conductance index.

**CO2 reactivity.** A representative example of blood pressure, MCAV, and expired CO2 changes during CO2 reactivity testing for one subject is shown in Fig. 1. The cardiovascular and respiratory responses to 5% CO2 inhalation and voluntary hyperventilation are summarized in Table 2. Inhalation of 5% CO2 and subsequent hyperventilation resulted in marked increases and decreases in \( \text{PETCO}_2 \), respectively (Table 2). The resultant hypercapnia and hypocapnia consistently altered MCAV mean and CVCi, and inconsistently altered MAP or HR (Table 2). The magnitude of hypercapnia was similar between the \( \alpha_1 \)-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 hypcapnia 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 \( \alpha_1 \)-adrenergic blockade were similar to each other (before vs. after) and similar to placebo.

Linear mixed-effects analysis showed that typically \( \text{PETCO}_2 \) and MAP were both significant predictors of MCAV mean dynamics, justifying the inclusion of MAP as a covariate. The effects of \( \alpha_1 \)-adrenergic blockade or placebo on integrated, hypercapnic, and hypocapnic CO2 reactivity are summarized in Table 3 and in Fig. 2, which highlights the magnitude of the treatment effects. Integrated, hypocapnic, and hypercapnic CO2 reactivity in both the \( \alpha_1 \)-adrenergic blockade and placebo groups were reduced following treatment (Fig. 2). However, three-way interaction (group \( \times \) treatment \( \times \) CO2) effects were observed only for the integrated and hypocapnic response, indicating
Table 3. Effects of α1-adrenergic blockade and placebo on CO2 reactivity

<table>
<thead>
<tr>
<th>CO2 Reactivity, cm s(^{-1}) mmHg(^{-1})</th>
<th>α1-Adrenergic Blockade</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
</tr>
<tr>
<td>Integrated response</td>
<td>2.5 ± 0.14</td>
<td>2.5 ± 0.14†</td>
</tr>
<tr>
<td>Hypocapnic response</td>
<td>1.9 ± 0.17</td>
<td>2.4 ± 0.16</td>
</tr>
<tr>
<td>Hypercapnic response</td>
<td>2.7 ± 0.15</td>
<td>3.0 ± 0.15</td>
</tr>
</tbody>
</table>

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

that, after accounting for time-controlled changes (placebo group), α1-adrenergic blockade blunted integrated and hypocapnic CO2 reactivity but not the CO2 reactivity response to hypercapnia (Fig. 2). No significant main effects or interactions were found for the integrated, hypercapnic, or hypocapnic CO2 reactivity delays (Table 4), indicating that α1-adrenergic blockade did not alter CO2 reactivity latency.

DISCUSSION

The main findings of this study were that α1-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 CO2 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 α1-adrenergic activity on CO2 reactivity is limited to the magnitude and not the latency of the response. Furthermore, hypercapnic and hypocapnic CO2 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 CO2 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 CO2 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 α1-adrenergic receptor blockade blunted CO2 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 α1-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 α1-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 CO2.

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 CO2-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 CO2 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 CO2. If confirmed, our findings may help explain why sympathetic dysfunction is associated with adverse cerebrovascular outcomes.

While our findings implicate the sympathetic system in CO2 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 CO2 reactivity to hypocapnia by ~26%. Therefore, hypocapnia-induced vasoconstriction appears to be largely driven independently of α1-adrenergic receptor stimulation within the ranges of PETCO2 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., KATP) leading to depolarization of the cell membrane, an increase in cytosolic Ca2+, and reduction in vessel caliber (29). This notion is based on studies implicating the reciprocal response during hypercapnic vasodilation (13, 22). Another possibility is that alterations in vasoactive factors play a role in hypocapnic cerebral vasocon-

[Fig. 2. Effect of α1-adrenergic blockade or placebo on integrated, hypocapnia, and hypercapnia CO2 reactivity (cm s\(^{-1}\) mmHg\(^{-1}\)). Bars show the change scores following treatment. *P < 0.05 prazosin vs. placebo. For hypercapnia, P = 0.50.]
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 $P_{\text{aCO}_2}$ in healthy humans. They found similar levels of endothelin-1, NO metabolites, and adrenomedullin during air breathing and graded hypocapnia down to 24 mmHg $P_{\text{ETCO}_2}$, 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, $\alpha_1$-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 conceivably given that $\alpha_1$-adrenergic stimulation causes vascular smooth muscle constriction rather than dilatation. Our results do not negate the potential for sympathetic activity to effect vasodilatation via $\beta$-adrenergic receptor stimulation, although previous studies (14, 23, 45) have consistently failed to demonstrate an effect of $\beta$-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 $\alpha_1$-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 $P_{\text{ETCO}_2}$ rather than the cerebrovascular response to steady-state changes in $P_{\text{ETCO}_2}$ (21, 25). Steady-state CO$_2$ reactivity reflects the net effect of all mechanisms engaged by changes in $P_{\text{aCO}_2}$ 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 $\alpha_1$-adrenergic receptor blockade, indicating that the putative influence of $\alpha_1$-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 MCA$_V$ 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 $P_{\text{ETCO}_2}$ 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 $P_{\text{ETCO}_2}$. 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 effenter cerebral sympathetic outflow is differentially regulated from effenter 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 $P_{\text{ETCO}_2}$.

### Table 4. Effects of $\alpha_1$-adrenergic blockade and placebo on CO$_2$ reactivity latency

<table>
<thead>
<tr>
<th>Latency, s</th>
<th>$\alpha_1$-Adrenergic Blockade</th>
<th>Placebo</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>Pretreatment</td>
</tr>
<tr>
<td>Integrated</td>
<td>13 ± 2.3</td>
<td>11 ± 3.0</td>
<td>13 ± 2.8</td>
</tr>
<tr>
<td>Hypocapnic</td>
<td>12 ± 1.9</td>
<td>9.1 ± 2.6</td>
<td>10 ± 3.0</td>
</tr>
<tr>
<td>Hypercapnic</td>
<td>14 ± 2.1</td>
<td>12 ± 4.0</td>
<td>14 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in seconds.
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 CO2 reactivity via α1-adrenergoreceptors as blocking this pathway with prazosin reduced CO2 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 CO2 reactivity.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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