Human cerebral arteriovenous vasoactive exchange during alterations in arterial blood gases

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Submitted 7 May 2008; accepted in final form 8 July 2008

Cerebral blood flow (CBF) is highly regulated by changes in arterial Pco2 and arterial Po2. Evidence from animal studies indicates that various vasoactive factors, including release of norepinephrine, endothelin, adrenomedullin, C-natriuretic peptide (CNP), and nitric oxide (NO), may play a role in arterial blood gas-induced alterations in CBF. For the first time, we directly quantified exchange of these vasoactive factors across the human brain. Using the Fick principle and transcranial Doppler ultrasonography, we measured CBF in 12 healthy humans at rest and during hypercapnia (4 and 8% CO2), hypocapnia (voluntary hyperventilation), and hypoxia (12 and 10% O2). At each level, blood was sampled simultaneously from the internal jugular vein and radial artery. With the exception of CNP and NO, the simultaneous quantification of norepinephrine, endothelin, or adrenomedullin showed no cerebral uptake or release during changes in arterial blood gases. Hypercapnia, but not hypocapnia, increased CBF and caused a net cerebral release of nitrite (a marker of NO), which was reflected by an increase in the venous-arterial difference for nitrite: 57 ± 18 and 150 ± 36 μmol/l at 4% and 8% CO2, respectively (both P < 0.05). Release of cerebral CNP was also observed during changes in CO2 (hypercapnia vs. hypocapnia, P < 0.05). During hypoxia, there was a net cerebral uptake of nitrite, which was reflected by a decreased venous-arterial difference for nitrite: −96 ± 14 μmol/l at 10% O2 (P < 0.05). These data indicate that there is a differential exchange of NO across the brain during hypercapnia and hypoxia and that CNP may play a complementary role in CO2-induced CBF changes. 

Catalyzed by the enzyme NO synthase (NOS) (48), NO is an important vasoactive factor that dilates the cerebral vasculature. Under normal conditions, two isoforms of NOS are expressed in the brain, endothelium-derived NOS (eNOS) and neuronal-derived NOS (nNOS) (61), although evidence for the importance of NO reported in previous human studies using pharmacological blocking agents have been widely used to examine the role of NO in arterial blood gas-induced CBF changes. In a number of studies, the cerebral vasodilatory response to hypercapnia and hypoxia has been inhibited by l-arginine analogs (30, 63), suggesting the involvement of NO; however, results have not been consistent, potentially because of differences in the sensitivity to NOS inhibitors within and between species, including humans (56, 66). This problem is compounded in human-based studies, where the extent to which NO inhibitors are able to cross the blood–brain barrier is unclear (23, 56, 66). Consequently, it is possible that the importance of NO reported in previous human studies using NO inhibitors is underestimated, and no studies have examined the potential exchange of NO across the human brain. It should be acknowledged, however, that NO is unlikely to play an exclusive role in the CBF response to hypercapnia, inasmuch as a residual increase in CBF is seen after NO inhibition (66). Furthermore, it seems unlikely that reduction in NO plays a major role in the CBF response to hypoxia in humans, since NO inhibition does not influence the cerebral vasoconstrictor response to hypoxia in animals (63).

In addition to NO, the cerebral vasculature is also influenced by a range of other vasoactive factors, including potent vasodilators such as adrenomedullin (ADM) (38) and C-type natriuretic peptide (CNP) (53) and vasoconstrictors such as endothelin-1 (ET-1) (20) and catecholamines, which can function as vasoconstrictors or vasodilators, depending on dosage and receptor expression (18). Although other local factors may also influence CBF during such changes in arterial blood gases (3, 6, 9), the aforementioned vasoactive factors, including NO (as indexed by nitrite), are reliably quantifiable in human blood. Surprisingly, however, none of these vasoactive substances have been sampled across the human brain during controlled changes in arterial blood gases.

Evidence for a potential involvement of ADM, CNP, and endothelin in CBF changes during hypoxia is supported by animal and cultured cell studies showing that ADM (55), CNP
its stable metabolites, nitrite and NOx (nitrite and conformed to the standards set by the Declaration of Helsinki. All studies that have been completed (26, 60) have only considered the role of individual vasoactive agents and only provide an indirect measure of cerebral levels, since blood was procured from peripheral venous samples. Consequently, there is no information that correlates the effect of a variety of vasoactive factors during acute changes in CBF across the human brain. Given the uncertainties regarding endogenous vasoactive agents involved in human CBF regulation during blood gas changes, the main purpose of the present study was to quantify the level of NO and the other aforementioned vasoactive agents in cerebrovascular changes during a series of controlled alterations in $P_{\text{aCO}}$, $P_{\text{aO}}$, and to explore correlations between these agents and CBF.

We elected to quantify the contribution of NO by measuring its stable metabolites, nitrite and NOx (nitrite + nitrate), simultaneously from arterial and internal jugular venous blood to provide insight into NO exchange in the human brain. In addition, we also quantified the potential influence of ADM, CNP, ET-1, and catecholamines (epinephrine and norepinephrine) during blood gas-induced CBF alterations. We hypothesized that hypercapnia and hypoxia, but not hypocapnia, would primarily result in a progressive release of nitrite and NOx from the brain. We also hypothesized that other vasoactive factors would, to a lesser extent, contribute during these alterations in blood gases, specifically, that an increase in the vasodilators ADM, CNP, and catecholamines and a decrease in the vasoconstrictor ET-1 would potentially contribute to an increase in CBF during hypercapnia. Moreover, an increase in ADM, CNP, and catecholamines would contribute to a potential elevation in CBF during hypoxia, although the magnitude of the vasodilation may be offset by an increase in ET-1.

**MATERIALS AND METHODS**

**Subjects**

Twelve healthy individuals [27 ± 5 (SD) yr of age, 24 ± 4 kg/m² body mass index, 10 men and 2 women] volunteered for the study, which was approved by the Lower South Regional Ethics Committee and conformed to the standards set by the Declaration of Helsinki. All participants received verbal and written explanation of the experimental procedures, including risks involved in the study, and written informed consent was obtained. Participants were nonsmokers and were not on any medication, and none had a known history of cardiovascular, neurological, or respiratory disease. To minimize the potential effect of estrogen on vascular function, the two female subjects were studied during the early follicular phase of their menstrual cycle.

**Experimental Design**

After full familiarization with the experimental protocol (excluding cannulation), participants arrived at the laboratory (>1 wk) having abstained from exercise and alcohol for 24 h and having not consumed a heavy meal or items containing caffeine for 4 h.

**Experimental Protocol**

**Cannulation of internal jugular vein and radial artery.** After placement of electrocardiogram leads and a peripheral O₂ saturation monitor, participants were positioned in the Trendelenberg position for placement of the internal jugular vein catheter under local anesthesia (1% lidocaine). A 16-gauge, 5-in. catheter (Arrow International) was advanced to the right jugular bulb using the Seldinger technique, and the position was confirmed by ultrasonography (Sonos 2000, Hewlett-Packard). Another catheter (20-gauge, BD Insite) was placed, under local anesthesia, into the radial artery. Both catheters were regularly infused with normal saline (0.9% NaCl) to maintain patency. After cannulation, participants rested quietly in the supine position, breathing room air while the monitoring equipment was placed (see below). During this time, the participants also acclimated to the facemask; after acclimatization, baseline measurements were obtained. Participants then underwent tests of cerebral vasoreactivity to CO₂ and O₂ (see below).

**Incremental and decremental CO₂ protocol.** Incremental hypercapnia was induced by switching the inspired gas from room air to 4% CO₂ (in 21% O₂-balance N₂, Hypercapnia I) for 4 min and then to 8% CO₂ (in 21% O₂-balance N₂, Hypercapnia II) for 4 min. The end-tidal $P_{\text{ETCO₂}}$ was also recorded during the final 30 s of each hypercapnic exposure. After the incremental hypercapnia, air breathing was allowed to ensure that baseline measurements were once again obtained. Participants were then instructed to hyperventilate by increasing their rate and depth of breathing to generate two levels (4-min at each level) of decremental hypocapnia. These levels matched, in an equal but opposite direction, the rise in $P_{\text{ETCO₂}}$ during the incremental hypercapnia steps, i.e., 4% and 8% CO₂. Training for hyperventilation was performed during the familiarization session, during which verbal feedback was provided to assist subjects in reaching and maintaining the target levels of hyperventilation.

**Incremental O₂ protocol.** Incremental hypoxia was induced by switching the inspired gas from room air to 12% O₂ (Hypoxia I) for 4 min and then to 10% O₂ (Hypoxia II) for 4 min. After the incremental hypoxia, air breathing was allowed, so that baseline measurements could once again be obtained.

**Monitoring equipment.** CBF velocity in the right middle cerebral artery (MCA) was measured using a 2-MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling, VA) and search techniques described elsewhere (1). In our laboratory, the day-to-day reproducibility [coefficient of variation (CV)] of mean cerebral artery blood flow velocity (MCAv) is 4.5%, which is consistent across the CO₂ ranges used in the present study (11, 49). Beat-to-beat arterial blood pressure and heart rate (HR) were monitored using finger photoplethysmography (Finometer, TPD Biomedical Instrumentation) and electrocardiography (Bio Amp, ADInstruments), respectively.

$P_{\text{ETCO₂}}$ was sampled from a leak-free mask and measured by a gas analyzer (model CD-3A CO₂ analyzer, AEI Technologies, Pittsburgh, PA). All data were acquired continuously at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML795, ADInstruments, Colorado Springs, CO) interfaced with a computer. Data were sampled at 200 Hz and stored for subsequent analysis using commercially available software (Chart version 5.02, ADInstruments).
Analytic Measurements

Blood samples were drawn simultaneously from the arterial and jugular catheters twice (separated by 10 min) during baseline air breathing and during the final 30 s of each of the hypercapnic and hypocapnic challenges. Before samples were procured, 1–2 ml of arterial and venous blood was aspirated from the catheter’s dead space and discarded. Arterial and venous blood was then drawn slowly over a 20-s period using 20-gauge cannulas to ensure adequate flow and avoid hemolysis. Blood to be analyzed for NO metabolites was transferred to chilled vacutainers containing lithium heparin (0.9% NaCl with ~5 IU heparin/ml whole blood). Blood to be analyzed for ADM, ET-1, catecholamines, and NH2-terminal peptide for CNP (NT-CNP) was transferred to chilled vacutainers containing EDTA. The contents of these vacutainers were immediately centrifuged at 4°C and separated, and the plasma was frozen at −80°C for later batch analysis. Immediately after acquisition, a small portion of the arterial and venous blood was transferred to capillary tubes for measurement of arterial and venous blood gases (NPT7 series, Radiometer). A total of ~200 ml of blood was drawn from each subject over the course of the experiment.

Plasma nitrite concentration was determined using a triiodide/ozone-based chemiluminescence assay, as described elsewhere (50). Each of the plasma samples was divided into two aliquots: one was injected into the reaction mixture [potassium iodide (45 mmol/l) and iodine (10 mmol/l) in glacial acetic acid] at 60°C, where it was purged with a helium stream in the NO chemiluminescence analyzer (Sievers Instruments, Boulder, CO); the other was treated with 1:10 (vol/vol) 5% sulfuramidine in 1 M HCl to scavenge nitrite for 20 min before injection. The difference in the two peaks provides an indication of plasma nitrite concentration. Plasma nitrate concentration was assessed after enzymatic reduction to nitrite (with vanadium chloride in HCl at 94°C as the reductant) using a flow-injection analysis based on the Griess reaction. All samples were tested in duplicate, and the intra- and interassay CVs of nitrite and nitrate were <2% and <3%, respectively.

Established methods were used to measure ADM (38), ET-1 (31), and catecholamines (15, 17). ADM was measured by radioimmunoassay, as described by Lewis et al. (38). Briefly, 1 ml of EDTA-containing plasma was applied to SepPak C18 cartridges and eluted with 80% isopropanol-0.013 M HCl. The eluant was dried under air and reconstituted with buffer containing 0.1% alkali-treated casein and assayed by radioimmunoassay. Standards or samples were incubated overnight at 4°C with ADM antiserum (50 μl) and 2,500 cpm of 125I-labeled ADM. Antibody-bound ADM was separated from unbound ADM by centrifugation in the presence of a second antibody. The supernatant was aspirated, radioimmunoreactivity of the pellet was measured in a gamma counter, and results were interpolated from an ADM standard curve. Using these techniques, Lewis et al. reported a detection limit of 0.1 fmol/tube, an intra-assay CV of 5.7–8.2%, and an interassay CV of 8.0–26.7%. The mean plasma ADM concentration in 44 normal human volunteers was 6.09 ± 0.3 pmol/l (range 2.7–10.1 pmol/l) (38).

Plasma immunoactive ET-1 was measured using an acetic acid extraction technique and a modified commercial radioimmunoassay using rabbit antihuman ET-1 (Peninsula Laboratories Europe, Sheffield, UK) (31). Briefly, 2 ml of EDTA-containing plasma were applied to SepPak C18 cartridges and eluted with 80% isopropanol in 0.1% TFA, dried under air, and reconstituted with 0.5 ml of 0.1 M phosphate buffer. Endothelin was assayed by addition of 100 μl of standard or extract containing endothelin antiserum (catalog no. RAS6901, Bachem). The assay was incubated for 3 h at room temperature. After incubation, 125I-labeled ET-1 (NEN Life Science Products, Boston, MA) was added, and incubation was continued for an additional 22–24 h at 4°C. Complexes were precipitated with Amerlex donkey anti-rabbit antibody (Amersham Life Sciences, Little Chalfont, Bucks, UK) and counted for radioactivity. All endothelin values were expressed as picograms per milliliter. Intra- and interassay CVs were 12.7% at 1.8–3.0 pmol/l and 12.5% at 3.0 pmol/l, respectively, and the sensitivity of the assay for ET-1 was 0.25 pg/ml. The mean for the assay in 200 normal human volunteers in our laboratory was 0.9 ± 2.3 pmol/l.

For the catecholamine assay, plasma was extracted on alumina, and catecholamines were eluted with acetic acid using established techniques (15, 17). The extracted catecholamines were separated and measured by reverse-phase high-performance liquid chromatography with electrochemical detection. The reference range for 45 healthy subjects reported by our laboratory is 47–3,800 pmol/l for norepinephrine and <570 pmol/l for epinephrine. The intra- and interassay CVs were <6% for both.

Since plasma CNP levels are very low and, therefore, difficult to measure, we used the NT-CNP assay as an index of CNP. NT-CNP is co-secreted with CNP and provides a sensitive and reliable alternative (53). The NH2-terminal propeptide for CNP (NT-proCNP) was assayed as previously described (52). Two milliliters of plasma were extracted on SepPak C18 cartridges and eluted with 2 ml of 80% isopropanol in 0.1% TFA, dried under air, and reconstituted with 0.5 ml of phosphate buffer. The extracted NT-CNP was added to primary rabbit antiserum (J39) raised against NT-proCNP-(1-15) (1:6,000 dilution, 100 μl antiserum/assay tube). Peptide standards were made from synthetic human proCNP-(1-15), with the purity data supplied by the manufacturer (Chiron Technologies) taken into account. Within- and between-assay CVs were 6.0% and 7.9%, respectively.

Nitrite and NOx were quantified during air breathing and at each of the CO2 and O2 levels. Because of ethical constraints regarding the volume of blood sampled and the large amount of plasma required for the various assays, ADM, ET-1, NT-CNP, and catecholamines were only quantified during air breathing and during the most severe levels of hypercapnia (8% CO2), hypocapnia (Hypocapnia II), and hypoxia (10% O2).

Calculations

Cerebral arterial and venous O2 contents (CaO2 and CVo2, respectively) were calculated using standard equations used by Hijazi et al. (21) and Stringer et al. (58) as follows:

\[
CaO_2 = 1.34 \cdot Hb \cdot (SaO_2) + 0.003 (PaO_2)
\]

(1)

\[
CV_o_2 = 1.34 \cdot Hb \cdot (SvO_2) + 0.003 (PvO_2)
\]

(2)

where SaO2 and SvO2 represent arterial and venous O2 saturation and PV02 is venous PO2. CBF was determined using the Fick equation as used by Kety and Schmidt (29):

\[
CBF = CMRO_2/CaO_2 - CVo_2
\]

(3)

where CMRO2 is cerebral metabolic rate of O2. Since previous human studies showed CMRO2 to be unchanged with alterations in PaCO2 and PaO2, comparable with those measured in the present study, a stable CMRO2 of 3.2 ml·100 g−1·min−1 was assumed (28, 65).

Exchange of endothelial markers. The exchange of nitrite and NOx and other endothelial markers over the brain was initially calculated by subtracting the venous concentration of each respective marker from the arterial concentration (v-a), where a positive v-a reflects a net release or outflow of a given biomarker and a negative v-a signifies net uptake or consumption. If the v-a was significantly different from baseline air breathing, as in the case of nitrite and NOx, the cerebral balance was also calculated: the cerebral balance of nitrite and NOx was calculated as cerebral plasma flow multiplied by the cerebral venous-arterial nitrate or NOx difference, where cerebral plasma flow was calculated on the basis of global CBF [estimated assuming an average brain mass of 1,400 g (13)] and the corresponding hematocrit value. Again, a positive value reflects net release and a negative value signifies net uptake.

J Appl Physiol • VOL 105 • OCTOBER 2008 • www.jap.org
Statistical Analysis

All data were analyzed using the social statistics package (version 12) from SPSS (Surrey, UK). One-way ANOVA was used to assess the statistical differences following interventions, with the Bonferroni-Dunn test used for post hoc analysis when a significant effect was found. A Pearson correlation analysis was used to determine the relationship between selected variables. All group data are expressed as means ± SD. Significance was established at an alpha level of P < 0.05.

RESULTS

Incremental and Decremental CO2 Protocol

Incremental hypercapnia caused a progressive increase in PaCO2, MCAv, and CBF, whereas incremental hypocapnia caused a progressive decline in PaCO2, MCAv, and CBF (Tables 1 and 2). Despite a significant correlation between MCAv and CBF (r = 0.86, P < 0.05), indicating that MCAv provides a reliable index of CBF, during 8% CO2 the relative increase in CBF was ~2.5-fold greater than baseline (as determined using the Fick principle) and the relative increase in MCAv was ~1.5-fold greater (as determined using Doppler ultrasonography) (Table 1). There was no significant change in mean blood pressure or HR during incremental hypercapnia or hypocapnia (Table 1).

Exchange of Vasoactive Factors

During incremental hypercapnia, but not hypocapnia, there was an increase in v-adiff for nitrite, indicating a marked release of nitrite from the brain (Table 3). A similar pattern was seen for the cerebral v-adiff for NOx, although the individual data reveal more variability (Table 3). The cerebral balance data reveal similar changes. For example, MCAv was correlated with the net cerebral nitrite balance (r = 0.78, P < 0.05) and net cerebral NOx balance (r = 0.54, P < 0.05). However, independent analysis of the hypercapnic and hypocapnic data revealed a significant relationship between MCAv and net cerebral nitrite (r = 0.70, P < 0.05), but not NOx (r = 0.44, NS), in the hypercapnic range; no correlations were evident between MCAv and net cerebral nitrite or NOx in the hypocapnic range (Fig. 1).

The v-adiff for epinephrine, norepinephrine, ET-1, NT-CNP, and ADM was unchanged between air breathing and 8% CO2 (Table 3). There was no significant change in mean blood pressure or HR during incremental hypercapnia or hypocapnia (Table 1).

Table 1. Effect of air breathing, hypercapnia, hypocapnia, and hypoxia on systemic and cerebral hemodynamics

<table>
<thead>
<tr>
<th>Condition</th>
<th>MCAv, cm/s</th>
<th>CBF, ml·min⁻¹·100 g⁻¹</th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>61.0 ± 14.9</td>
<td>60.0 ± 18.8</td>
<td>87.5 ± 9.2</td>
<td>59.2 ± 8.5</td>
</tr>
<tr>
<td>Hypcapnia I</td>
<td>70.9 ± 16.2</td>
<td>75.7 ± 31.3</td>
<td>88.7 ± 8.9</td>
<td>59.5 ± 8.4</td>
</tr>
<tr>
<td>Hypcapnia II</td>
<td>106.7 ± 22.5*</td>
<td>147.5 ± 58.6*</td>
<td>98.4 ± 10.6</td>
<td>58.7 ± 3.6</td>
</tr>
<tr>
<td>Hypcapnia I</td>
<td>55.7 ± 21.1</td>
<td>52.9 ± 19.6</td>
<td>86.1 ± 8.8</td>
<td>58.1 ± 5.1</td>
</tr>
<tr>
<td>Hypcapnia II</td>
<td>40.3 ± 12.1*</td>
<td>32.4 ± 4.7*</td>
<td>79.9 ± 15.5</td>
<td>59.1 ± 6.9</td>
</tr>
<tr>
<td>Hypoxia I</td>
<td>69.9 ± 21.1</td>
<td>55.9 ± 12.2</td>
<td>90.6 ± 12.6</td>
<td>69.6 ± 6.5*</td>
</tr>
<tr>
<td>Hypoxia II</td>
<td>72.3 ± 21.5</td>
<td>59.4 ± 12.0</td>
<td>80.4 ± 12.6</td>
<td>83.4 ± 10.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD for 12 subjects. Hypoxia I and II, 4% and 8% CO2 (in 21% O2-balance N2), respectively; Hypcapnia I and II, decremental hypoxia at levels that matched, in an equal but opposite direction, rise in end-tidal PCO2 during incremental hypoxemia; Hypoxia I and II, 12% and 10% O2; MCAv, mean cerebral blood flow velocity; CBF, cerebral blood flow; MABP, mean arterial pressure; HR, heart rate. *Significantly different from air (P < 0.05). †Significantly different from hypercapnia I (P < 0.05).

DISCUSSION

The major findings of the present study are as follows. 1) Compared with baseline, there was no cerebral uptake or release of other vasoactive factors (ADM, ET-1, NT-CNP, and catecholamines) during blood gas alterations; however, when the full range of CO2 stimuli (i.e., from Hypcapnia II to 8% CO2) was examined, there was a relative increase in release of NT-CNP from the brain with progressive hypercapnia. 2) There was a marked release of nitrite from the brain during hypercapnia-induced elevations in CBF but no changes during hypcapnia-induced decreases in CBF. 3) In contrast to our original hypothesis, although CBF was unchanged in acute hypoxia, there was a selective uptake of nitrite in the brain. On the basis of our experimental approach, although we acknowledge the limitation of correlational analysis (which does not necessarily mean cause and effect), these findings indicate a differential exchange of NO during hypercapnia and hypoxia.
across the human brain. On the basis that NT-CNP is a reliable index of CNP, our data indicate that CNP release from the brain may also play a supplemental role in regulating CBF during changes in $P_{\text{CO}_2}$.

$P_{\text{CO}_2}$ and Nitrite Exchange

Calculation of NO exchange kinetics directly across the human brain via simultaneous measurement of arterial and internal jugular venous blood provided, for the first time, evidence of a net release of nitrite from the brain during hypercapnia, whereas no changes were evident during hypocapnia. These changes occurred without the confounding influence of pharmacological agents. Within the brain, NO is produced in the endothelium, as well as in neuronal tissue, i.e., neurons, perivascular nerves, and astrocytes. In pigs, hypercapnia provokes an increase in CBF and in eNOS mRNA expression; both were blunted by a nonselective NOS inhibitor, but not by a selective nNOS inhibitor (45), suggesting that the CBF increase is associated with an increase in endothelial, rather than neuronal, NO. Conversely, in rodents, neuronal NO was the more important source (47). Collectively, although the precise contribution of eNOS and nNOS during hypercapnia is unclear (45), previous animal and human studies indicate that eNOS is an important, although not exclusive, mediator of hypercapnia-induced alterations in CBF in healthy young humans (36). It should be noted that the index of NO used in the present study was confined to eNOS, yet the observed increase in CBF represents a collective response. Given the wide variety of sources of cerebral NO, we cannot exclude the possibility that our nitrate measurements may underestimate the true release

### Table 2. Effect of air breathing, hypercapnia, hypocapnia, and hypoxia on blood gas parameters

<table>
<thead>
<tr>
<th>Condition</th>
<th>$n$</th>
<th>pH</th>
<th>$PCO_2$, Torr</th>
<th>$PO_2$, Torr</th>
<th>$O_2$ Saturation, %</th>
<th>pH</th>
<th>$PCO_2$, Torr</th>
<th>$PO_2$, Torr</th>
<th>$O_2$ Saturation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>12</td>
<td>7.44 ± 0.03</td>
<td>39 ± 4</td>
<td>112 ± 10</td>
<td>99 ± 1</td>
<td>7.39 ± 0.05</td>
<td>50 ± 6</td>
<td>37 ± 5</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>Hypercapnia I</td>
<td>9</td>
<td>7.40 ± 0.06</td>
<td>43 ± 5</td>
<td>131 ± 10</td>
<td>100 ± 1</td>
<td>7.35 ± 0.05</td>
<td>52 ± 4</td>
<td>41 ± 9</td>
<td>76 ± 8</td>
</tr>
<tr>
<td>Hypercapnia II</td>
<td>12</td>
<td>7.35 ± 0.05*</td>
<td>53 ± 5*†</td>
<td>136 ± 7*</td>
<td>99 ± 0</td>
<td>7.33 ± 0.04</td>
<td>56 ± 8</td>
<td>58 ± 9*†</td>
<td>87 ± 2*†</td>
</tr>
<tr>
<td>Hypocapnia I</td>
<td>11</td>
<td>7.47 ± 0.03</td>
<td>33 ± 5</td>
<td>129 ± 13*</td>
<td>100 ± 1</td>
<td>7.41 ± 0.05</td>
<td>44 ± 10</td>
<td>37 ± 11</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Hypocapnia II</td>
<td>9</td>
<td>0.57 ± 0.05*‡</td>
<td>724 ± 4*‡</td>
<td>134 ± 7*</td>
<td>100 ± 0*</td>
<td>7.44 ± 0.07</td>
<td>41 ± 7*</td>
<td>27 ± 7*</td>
<td>52 ± 13*‡</td>
</tr>
<tr>
<td>Hypoxia I</td>
<td>8</td>
<td>7.44 ± 0.04</td>
<td>36 ± 2</td>
<td>55 ± 19*</td>
<td>89 ± 6*</td>
<td>7.40 ± 0.04</td>
<td>46 ± 3</td>
<td>29 ± 3*</td>
<td>57 ± 5*</td>
</tr>
<tr>
<td>Hypoxia II</td>
<td>11</td>
<td>7.47 ± 0.07</td>
<td>35 ± 6</td>
<td>43 ± 19*</td>
<td>78 ± 10*§</td>
<td>7.41 ± 0.09</td>
<td>44 ± 7</td>
<td>26 ± 2*</td>
<td>50 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, number of subjects. See Table 1 footnote for explanation of conditions. *Significantly different from air ($P < 0.05$). †Significantly different from Hypercapnia I ($P < 0.05$). §Significantly different from Hypocapnia I ($P < 0.05$). ‡Significantly different from Hypoxia I ($P < 0.05$).

### Table 3. Arterial and venous vasoactive factors during air breathing, hypercapnia, and hypocapnia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Air</th>
<th>Hypercapnia I</th>
<th>Hypercapnia II</th>
<th>Hypocapnia I</th>
<th>Hypocapnia II</th>
<th>Hypoxia I</th>
<th>Hypoxia II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite, μmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>235 ± 74</td>
<td>241 ± 78</td>
<td>243 ± 77</td>
<td>237 ± 74</td>
<td>238 ± 75</td>
<td>241 ± 68</td>
<td>233 ± 76</td>
</tr>
<tr>
<td>Venous</td>
<td>240 ± 75</td>
<td>297 ± 79</td>
<td>398 ± 96*</td>
<td>241 ± 78</td>
<td>237 ± 73*</td>
<td>207 ± 82</td>
<td>137 ± 69</td>
</tr>
<tr>
<td>$\Delta$aff</td>
<td>3 ± 15</td>
<td>57 ± 18*</td>
<td>150 ± 36*†</td>
<td>7 ± 16</td>
<td>-1 ± 14§</td>
<td>34 ± 28</td>
<td>-96 ± 14§</td>
</tr>
<tr>
<td>NOx, mmol/l</td>
<td></td>
<td></td>
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<tr>
<td>Arterial</td>
<td>24 ± 7</td>
<td>24 ± 7</td>
<td>24 ± 7</td>
<td>24 ± 7</td>
<td>24 ± 7</td>
<td>25 ± 7</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Venous</td>
<td>27 ± 7</td>
<td>25 ± 7</td>
<td>27 ± 7</td>
<td>25 ± 7</td>
<td>25 ± 7</td>
<td>24 ± 7</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>$\Delta$aff</td>
<td>0 ± 2</td>
<td>1 ± 2</td>
<td>3 ± 2*</td>
<td>1 ± 2</td>
<td>1 ± 2</td>
<td>-1 ± 2</td>
<td>-2 ± 3</td>
</tr>
<tr>
<td>Epinephrine, pmol/l</td>
<td></td>
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<tr>
<td>Arterial</td>
<td>462 ± 280</td>
<td>968 ± 582*</td>
<td>387 ± 145§</td>
<td>1,024 ± 503*</td>
<td></td>
<td></td>
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<tr>
<td>Venous</td>
<td>434 ± 290</td>
<td>926 ± 572</td>
<td>335 ± 156§</td>
<td>966 ± 497*</td>
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<tr>
<td>$\Delta$aff</td>
<td>-28 ± 31</td>
<td>-41 ± 63</td>
<td>-9 ± 28</td>
<td>-59 ± 35</td>
<td></td>
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<tr>
<td>Norepinephrine, pmol/l</td>
<td></td>
<td></td>
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<tr>
<td>Arterial</td>
<td>1,986 ± 419</td>
<td>1,934 ± 659</td>
<td>1,919 ± 482</td>
<td>1,726 ± 448</td>
<td></td>
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<tr>
<td>Venous</td>
<td>2,093 ± 504</td>
<td>2,221 ± 691</td>
<td>2,306 ± 1,152</td>
<td>1,833 ± 752</td>
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<tr>
<td>$\Delta$aff</td>
<td>107 ± 392</td>
<td>287 ± 426</td>
<td>387 ± 1,000</td>
<td>107 ± 430</td>
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<tr>
<td>ET-1, pmol/l</td>
<td></td>
<td></td>
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<tr>
<td>Arterial</td>
<td>1.84 ± 0.41</td>
<td>1.85 ± 0.36</td>
<td>1.98 ± 0.57</td>
<td>1.94 ± 0.46</td>
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<tr>
<td>Venous</td>
<td>1.74 ± 0.23</td>
<td>1.99 ± 0.28</td>
<td>1.97 ± 0.49</td>
<td>1.99 ± 0.19</td>
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<tr>
<td>$\Delta$aff</td>
<td>-0.10 ± 0.48</td>
<td>0.15 ± 0.43</td>
<td>0.10 ± 0.83</td>
<td>0.06 ± 0.41</td>
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<tr>
<td>NT-CNP, pmol/l</td>
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<tr>
<td>Arterial</td>
<td>19.88 ± 3.90</td>
<td>24.33 ± 4.34</td>
<td>18.49 ± 3.96§</td>
<td>19.94 ± 4.24</td>
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<tr>
<td>Venous</td>
<td>22.42 ± 4.49</td>
<td>29.32 ± 4.65</td>
<td>20.34 ± 4.81§</td>
<td>24.7 ± 5.38</td>
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<tr>
<td>$\Delta$aff</td>
<td>2.53 ± 2.25</td>
<td>4.99 ± 2.55</td>
<td>1.84 ± 2.54§</td>
<td>4.80 ± 3.19</td>
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<tr>
<td>ADM, pmol/l</td>
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</tr>
<tr>
<td>Arterial</td>
<td>3.81 ± 1.30</td>
<td>4.35 ± 1.21</td>
<td>3.99 ± 1.15</td>
<td>3.91 ± 0.78</td>
<td></td>
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</tr>
<tr>
<td>Venous</td>
<td>5.09 ± 1.38</td>
<td>5.87 ± 1.65</td>
<td>5.26 ± 1.59</td>
<td>4.96 ± 1.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$aff</td>
<td>1.28 ± 1.88</td>
<td>1.51 ± 2.01</td>
<td>1.43 ± 1.85</td>
<td>1.04 ± 1.20</td>
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</table>

Values are means ± SD for 12 subjects. NOx, nitrate + nitrite; $\Delta$aff, venous-arterial difference; ET-1, endothelin-1; NT-CNP, NH$_2$-terminal C-natriuretic peptide; ADM, adrenomedullin. See Table 1 footnote for explanation of conditions. *Significantly different from air ($P < 0.05$). †Significantly different from Hypercapnia I ($P < 0.05$). ‡Significantly different from Hypocapnia I. §Significantly different from Hypercapnia II ($P < 0.05$).
of cerebral NO. In addition, we cannot exclude the potential role of a shear-stress response in mediating a release of NO (42). Because hypercapnia is associated with an increase in CBF and a concomitant release of plasma nitrite and because fluid shear stress evokes a stimulus-dependent increase in cerebral NO production (42), it seems reasonable to suggest that the progressive release of nitrite indicates, in part, a hypercapnia-induced shear-stress response and subsequent NO release. It was not the goal of the present study to separate these potential mechanisms; however, detailed animal studies are warranted to further delineate the influence of shear stress from hypercapnia-induced NO vasodilation.

Acute Hypoxia, CBF, and Nitrite Exchange

The observation that CBF did not increase during acute poikilocapnic hypoxia (i.e., where PETCO2 is uncontrolled) is consistent with previous human studies (2). The lack of an increase in CBF in the present study suggests that the cerebral vasconstriction induced by the fall in PaO2 was sufficient to counteract any vasodilatory effects of hypoxia. Contrary to our original hypothesis, there was a clear uptake of nitrite in acute hypoxia. There is only one human study (62) with which we can compare our findings: Van Mil et al. (62) reported an increase in CBF during 20 min of poikilocapnic hypoxia (80% O2 saturation measured by pulse oximetry) that was blunted by N-monomethyl-L-arginine, suggesting a hypoxia-induced release of NO. Our results are not consistent with this finding, although differences in methodological design, including the use of magnetic resonance imaging, a longer duration of hypoxia, and the use of an L-arginine analog, make direct comparison difficult. In support of our findings, in a recent study in pigs, Kirkeby et al. (30) employed an electronic NO sensor to directly measure cerebral NO concentration and reported a fall in NO concentration after 180 s of hypoxia. Since O2 is essential for the formation of NO via NOS, Kirkeby et al. proposed that the fall in NO was due to a hypoxia-induced reduction in NO synthesis.

It is possible that the level of hypoxia in the present study may not have been sufficient to elicit NO release. Nitrite has been considered an index of NO bioavailability, in particular, eNOS activity (33). Importantly, since nitrite can spontaneously decompose to NO when PaO2 levels are low (between ~40 and 20 Torr), it has been considered a “reservoir” for NO (16). We observed that, during the most severe hypoxic stimulus, PaO2 fell to 43 Torr (SaO2 ≈ 78%) and PvO2 to 26 Torr (SvO2 ≈ 50%; Table 3). The mechanism of action and location of the O2 sensor are not completely known but are likely downstream of the terminal arterioles (25). If the location of the O2 sensor was closer to the arterial than the venular end of the capillary network, it is possible that, at the point at which it was sensed, the level of PaO2 in the present study was not severe enough to stimulate NO release. Most importantly, it should be noted that NO is not the only source of nitrite in the blood. For example, it has been reported that red blood cells have functional eNOS and can produce NO (32). Moreover, NO binds avidly to Hb. Therefore, much of the NO entering the blood might be as HbNO, not as nitrite. Studies combining detailed measures of red blood cell- and Hb-derived nitrite levels are needed to clarify and extend our findings.

Role of Other Endothelial Markers During PaCO2 and PaO2 Changes

With the exception of CNP, during step changes in PCO2 or PO2, there was no cerebral release or uptake of ET-1, ADM, or catecholamines. Interestingly, a graded increase in arterial, venous, and v-αdiff for NT-CNP was apparent between hypoxia and hypercapnia (8% CO2). These findings indicate that the systemic and cerebral vasculature is able to release CNP in response to progressive hypercapnia, if the range of CO2 stimuli is sufficiently wide. Considerable physiological evidence suggests that vasoactive factors not only act independently but may also modulate each other. For instance, there are reports of complementary cross talk between CNP and NO in mounted tissue preparations (39). Undoubtedly, such interactions add to the complexity of analyzing vasoactive responses during CBF changes, and it was not the purpose of the present study to examine these in detail. However, during CO2 alterations, we saw parallel changes in cerebral nitrite release and cerebral NT-CNP release (P = 0.07). While we interpret this trend cautiously, it is of interest that NO and NT-CNP are derived from the endothelium (9, 39), the NO and CNP pathways are stimulated by shear stress (10, 42), and both substances were associated with cerebral vasodilation in response to increasing CO2. These commonalities, coupled with...
the aforementioned findings from mounted tissue preparations (39), suggest a cooperative interaction between the release of NO and CNP in the cerebral vasculature during CO2-induced blood flow changes that warrants further investigation.

Despite the absence of a v-\text{diff} for catecholamines, it is of interest that an increase in arterial and venous epinephrine was observed across the hypocapnic-to-hypercapnic gas range, as well as during the hypoxic interventions. We considered whether the carotid body might be involved in this response. The carotid bodies, apart from their fundamental role in the hypoxic ventilatory response (40), also play a role in the hypercapnic ventilatory response (12). Moreover, carotid body stimulation evokes an increase in sympathetic nerve activity in muscle and splanchnic and renal vascular beds and, thus, contributes to the significant interactions between ventilatory and cardiovascular control (40). These findings raise the possibility that the increase in systemic epinephrine might have occurred secondary to carotid body stimulation, although the absence of a simultaneous increase in norepinephrine is inconsistent with this suggestion.

**Methodological Considerations and Limitations**

The technique of measuring radial arterial-internal jugular venous differences provides important information regarding the net release and net uptake of vasoactive agents in the human brain. However, it is acknowledged that arteriovenous gradients do not provide information regarding the movement of vasoactive substances outside the blood circulation; e.g., our peptide markers only indirectly reflect levels in the cerebrospinal fluid and/or brain tissue. Furthermore, they do not provide insight as to whether a net increase in a given vasoactive agent is due to an increase in production/release or inhibition of uptake. It should be noted that the techniques used in the present study, i.e., arterial and venous cannulation, were invasive and represent a novel approach to quantification of cerebral vasoactive agents in conscious humans.

It has been widely accepted that the internal jugular venous system, rather than the external vertebral venous system, represents the major outflow for intracranial blood. However, several authors showed that venous outflow may be influenced by posture and anatomic variability (14, 54), raising the potential for nonjugular venous drainage to result in an underestimation of vasoactive agents. Unfortunately, these authors (14, 54) did not perform angiographic studies; however, Doepp et al. (14) reported that the internal jugular veins were the main source of venous drainage in 72% of their supine healthy volunteers.

It is recognized that the present study only quantified a selection of vasoactive factors as justified in the introduction. It was not possible to measure any additional factors, inasmuch as ethical constraints limit the total volume of blood that can be drained from human subjects. Although we used Doppler ultrasonography to measure flow velocity, rather than blood flow, in the MCA, the majority of research suggests that MCAv is a reliable index of CBF (51). In further support of this hypothesis, our data clearly show that the change in MCAv during related changes in P_{\text{ACO}_2} was closely related to the changes in global CBF (r = 0.86, P < 0.05) as estimated by the Fick principle. The difference between the relative increases in CBF and MCAv at 8% CO2 as estimated by the Fick principle and Doppler ultrasonography, respectively, warrants consideration. To the best of our knowledge, we believe that this is the first study to compare CBF as estimated by the Fick principle with CBF as estimated by Doppler ultrasonography. The reported increase in MCAv with hypercapnia (2–3% change per 1-Torr change in P_{\text{PETCO}_2}) is consistent with previous work from our laboratory and the work of others (43), whereas the changes in CBF as estimated by the Fick principle are entirely consistent with a CBF increase as estimated recently by the “gold-standard” pulsed arterial spin-labeling magnetic resonance method at 3 T (5.8% increase per 1 Torr) (46). One likely reason for this difference in Doppler ultrasound and Fick (and pulsed arterial spin labeling) estimations is that transcranial Doppler ultrasonography measures the blood velocity in the MCA, an area that transports blood to large brain volumes, including gray and white matter, whereas the pulsed arterial spin-labeling method measures the reactivity of small vessels and capillaries within a purely cortical gray matter area, where reactivity to CO2 is much higher (46).

Plasma nitrite and NOx were used as an index of NO. A major reason for choosing this technique is that it bypassed the use of NOS inhibitors, which may cause constriction in the MCA (66), potentially invaliding their use with transcranial Doppler ultrasonography. The specificity and sensitivity of plasma nitrite as an index of eNOS have been well demonstrated (33, 35). The lack of a detectable change in plasma venous-arterial NOx is not surprising, since it is influenced by a variety of NO synthase-independent factors, including dietary nitrate intake, formation of saliva, bacterial nitrate synthesis, denitrifying liver enzymes, inhalation of atmospheric gaseous NOSs, and renal function (59); thus the plasma NOx concentrations may not sensitively reflect acute changes in NO activity (35).

In the present study, the cerebrovascular responses to hypoxia were studied under poikilopcapnic conditions. It would be of interest to examine the hypoxia-induced changes in ventilation and CBF under isocapnic conditions, i.e., with P_{\text{ACO}_2} controlled, to remove the confounding influence of hypocapnia; however, since both hypocapnic conditions resulted in no change in the majority in the vasoactive substances, the influence of hypocapnia alone would seem not to have a major role in regulation of the quantifiable vasoactive substances across the brain.

In addition to NO, it should be noted that cerebrovascular CO2 reactivity is primarily mediated by pH and can also be modulated by cyclooxygenase activity (8). Interestingly, there is evidence of interactions between cyclooxygenase activity and NO. For example, several authors have shown that NO and prostacyclin, a cyclooxygenase derivative and an important dilator prostanoid in the cerebral circulation, act in a permissive manner; i.e., the hypercapnic vascular response to NO requires the presence of prostacyclin (37, 64). Given these findings, it was unfortunate that, because of lack of plasma and some doubts about the reliability of the use of a downstream metabolite as an index of prostacyclin, we were not able to make these additional measurements. Nevertheless, the relative roles of NO and prostanoids in hypercapnic cerebral dilation in humans may be age dependent, since there is evidence in pigs that the contribution of NO vs. prostacyclin to hypercapnic cerebrovascular dilation increases with age (67). In light of these findings, it is conceivable that significant increases in
CEREBRAL VASOACTIVE AGENTS AND ARTERIAL BLOOD GASES

prostacyclin during hypercapnic vasodilatation may not have been observed in the present study, since adult humans were studied. In conclusion, we have found that hypercapnia is associated with a net release of nitrite from the brain, whereas hypoxia is associated with a net uptake of nitrite. There was no net cerebral release or uptake of epinephrine, norepinephrine, ET-1, or ADMA during CO2 and O2 alterations; however, cerebral release of NT-CNP was evident when a wide range of CO2 alterations was examined. These data indicate that there is a differential exchange of NO across the brain during hypercapnia and hypoxia and that CNP may play a complementary role in CO2-induced CBF changes.

ACKNOWLEDGMENTS

We acknowledge the skilled technical assistance of Steve Fisher and the volunteers for their participation in this study.

GRANTS

This study was supported by the University of Otago Department of Physiology.

REFERENCES


