Transcerebral Exchange Kinetics of Nitrite and Calcitonin Gene-Related Peptide in Acute Mountain Sickness: Evidence Against Trigeminovascular Activation?

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Transcerebral Exchange Kinetics of Nitrite and Calcitonin Gene-Related Peptide in Acute Mountain Sickness

Evidence Against Trigeminovascular Activation?

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Background and Purpose—High-altitude headache is the primary symptom associated with acute mountain sickness, which may be caused by nitric oxide-mediated activation of the trigeminovascular system. Therefore, the present study examined the effects of inspiratory hypoxia on the transcerebral exchange kinetics of the vasoactive molecules, nitrite ($NO_2^{\bullet}$), and calcitonin gene-related peptide (CGRP).

Methods—Ten males were examined in normoxia and after 9-hour exposure to hypoxia (12.9% O_2). Global cerebral blood flow was measured by the Kety-Schmidt technique with paired samples obtained from the radial artery and jugular venous bulb. Plasma CGRP and $NO_2^{\bullet}$ were analyzed via radioimmunoassay and ozone-based chemiluminescence. Net cerebral exchange was calculated by the Fick principle and acute mountain sickness/headache scores assessed via clinically validated questionnaires.

Results—Hypoxia increased cerebral blood flow with a corresponding increase in acute mountain sickness and headache scores ($P<0.05$ vs normoxia). Hypoxia blunted the cerebral uptake of $NO_2^{\bullet}$, whereas CGRP exchange remained unaltered. No relationships were observed between the change (hypoxia–normoxia) in cerebral $NO_2^{\bullet}$ or CGRP exchange and acute mountain sickness/headache scores ($P>0.05$).

Conclusion—These findings argue against sustained trigeminovascular system activation as a significant event in acute mountain sickness. **(*Stroke*. 2009;40:2205-2208.)**

Key Words: acute mountain sickness ■ brain ■ calcitonin gene-related peptide ■ hypoxia ■ gene-related peptide ■ nitrite

High-altitude headache is the most common central nervous system complication that occurs in response to the inspiratory hypoxia of high altitude.[1,2] Recent evidence suggests that it is not directly attributable to mild high-altitude cerebral edema defined by vasogenic edematous brain swelling or intracranial hypertension.[3] The prophylactic benefits of sumatriptan[4] suggests that activation of the trigeminal vascular system may prove an alternative mechanism.

To explore this in more detail, the present study examined the effects of hypoxia on the cerebral metabolism of nitrite ($NO_2^{\bullet}$), an important intravascular reserve of nitric oxide (NO),[5] and calcitonin gene-related peptide (CGRP). These vasoactive molecules act synergistically to promote neurogenic vasodilatation and trigeminal vascular system activation, which is considered the primary mechanism responsible for vascular headaches at sea level.[6] We hypothesized that if NO-mediated trigeminal vascular system activation is implicated in the pathophysiology of acute mountain sickness (AMS), hypoxia would result in a cerebral uptake or consumption of $NO_2^{\bullet}$, thus increasing the bioavailable pool of NO. We further reasoned that this would stimulate the synthesis and net cerebral output of CGRP that would be positively related to corresponding symptom scores.

Materials and Methods

Subjects and Design

Ten healthy males aged 27 (mean)±4 (SD) years without any history of headache, migraine, or AMS provided written informed consent following approval by the Scientific Ethics Committee of Copenhagen and Frederiksberg Municipalities (Denmark). Catheters were placed under local anesthesia in the radial artery and the right internal jugular vein via ultrasound guidance with the tip pointing cranially. After 30 minutes of supine rest, measurements were obtained in normoxia (21% O_2), and measurements were obtained after 9 hours of passive exposure to normobaric hypoxia (12.9% O_2).

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Table 1. Cerebral Hemodynamics

Condition	Normoxia		Hypoxia	
Sample Site	Arterial	Venous	Arterial	Venous
PO_2 (mm Hg)	107±6	38±2*	46±3†	29±2*†
	Main effects for condition+sample site+interaction effect			
SO_2 (%)	99±1	66±2	83±3	51±5
	Main effects for condition+sample site			
PCO_2 (mm Hg)	43±2	53±2*	35±2†	44±2*†
	Main effects for condition+sample site+interaction effect			
CBF (mL/100g/min)	85±15		94±17	
Adjusted CBF (mL/100g/min)	85±15		123±24†	
CPF (mL/100g/min)	51±9		57±11	
Adjusted CPF (mL/100g/min)	51±9		74±16†	
O_2 delivery (μmol/g/min)	7.3±1.4		8.6±1.4	

CBF/CPF indicates global cerebral blood/plasma flow expressed in absolute values and adjusted for changes in $PaCO_2$; PO_2/PCO_2, partial pressure of oxygen/carbon dioxide; SO_2, oxyhemoglobin saturation.

*Different vs arterial for given condition ($P<0.05$).

†Different vs normoxia for given sample site ($P<0.05$).

Cerebral Hemodynamics

Hemoglobin (Hb), hematocrit (Hct), partial pressures of oxygen, carbon dioxide (Po_2/Pco_2), and oxyhemoglobin saturation (SO_2) were determined using a blood gas analyzer (ABL 715 Radiometer). Global cerebral blood flow (CBF) was measured using the Kety-Schmidt technique[7] in the desaturation mode using 5% nitrous oxide as the tracer with a coefficient of variation (CV) of 12%.[8] To normalize for the cerebral vasoconstrictive effects of hypocapnia during the hypoxic challenge, absolute CBF values were also adjusted for changes in $PaCO_2$:

$$\text{Adjusted CBF}_{\text{HYPOXIA}} = \frac{\text{measured CBF}_{\text{HYPOXIA}}}{[1+(\text{PaCO}_{2\text{HYPOXIA}}-\text{PaCO}_{2\text{NORMOXIA}})\times 0.03)]}$$

Global cerebral plasma flow (CPF) was calculated as CBF×(1−Hct), and cerebral O_2 delivery as CBF×arterial oxygen content [1.39 (Hb)×(SO_2/100)+(0.003×Po_2)].

Metabolic Measurements

Blood was centrifuged at 600*g* (4°C) for 10 minutes and K-EDTA plasma injected into tri-iodide reagent for the measurement of $NO_2^{\bullet}$ via ozone-based chemiluminescence with an intra and interassay CV of 7% and 10%, respectively (detection limit <1 nmol/L) based on data collected in healthy volunteers.[9] Plasma CGRP was analyzed using the most accurate, precise, sensitive, specific, and validated radioimmunoassay currently available with an intra and interassay CV of 2% and 7% (detection limit <1 pmol/L) based on healthy volunteers.[10,11] In our experience, these techniques yield normative (radial) arterial and (jugular) venous concentrations of $NO_2^{\bullet}$ and CGRP that range between ≈150 and 850 nmol/L and ≈50 and 100pmol/L at rest in normoxia, which is consistent with the published literature.[11,12] Cerebral net exchange was calculated as (unadjusted) CPF×arteriovenous concentration difference (a-v_{diff}).

Cephalalgia

AMS was assessed using the Lake Louise and Environmental Symptoms Questionnaire Cerebral scoring systems and a visual analogue scale was used to examine headache as previously described.[13]

Statistical Analysis

Shapiro-Wilk *W* tests confirmed distribution normality. Data were analyzed with a 2-way repeated measures analysis of variance (condition×sample site) with *post hoc* Bonferroni-corrected paired samples *t* tests. Relationships were analyzed using a Pearson Product Moment Correlation. Significance was established at $P<0.05$ and values are presented as mean±SD.

Results

Cerebral Hemodynamics

Hypoxia resulted in marked arterial hypoxemia as reflected by a reduction ($P<0.05$ vs normoxia) in PaO_2, SaO_2, and $PaCO_2$ (Table 1). Global CBF (absolute and $PaCO_2$-adjusted values) and corresponding CPF tended to increase, thus preserving cerebral O_2 delivery.

Cerebral Metabolic Exchange

Normoxia was associated with a positive a-v_{diff} and, hence, net cerebral influx or uptake of $NO_2^{\bullet}$ (Figure, A and B), whereas CGRP exchange was unremarkable (Figure, C and D). Hypoxia blunted the cerebral uptake of $NO_2^{\bullet}$ attributable primarily to a decrease in arterial delivery, whereas no changes were observed in systemic or cerebral CGRP metabolism.

Cephalagia

Hypoxia increased the severity of AMS and corresponding headache scores (Table 2), although these were unrelated to any of the changes observed in CBF ($r=0.26-0.44$; $P>0.05$) or cerebral exchange of NO_2 ($r=-0.42--0.14$, $P>0.05$) and CGRP ($r=-0.13-0.08$; $P>0.05$).

Discussion

These findings fail to support our original hypothesis and contrast with some of the observations reported in the migraine (without aura) literature, a complication of the central nervous system that shares phenotypic characteristics that are virtually indistinguishable from those encountered in AMS. Goadsby et al[14] were the first to document increased CGRP levels in the external jugular venous blood of patients during migraine attack, a finding that has since been replicated in cubital venous[15] and internal jugular venous[16] blood. Furthermore, CGRP infusion causes migraine-like head-

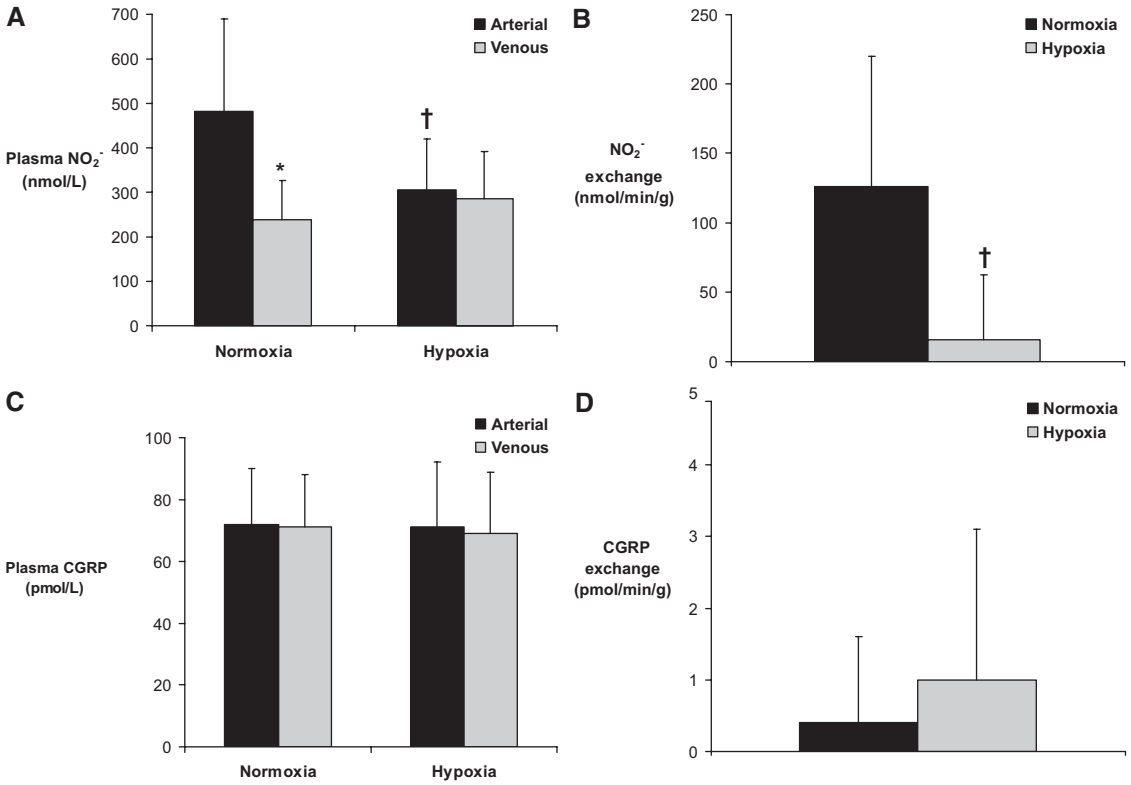

Figure. Arterio-jugular venous plasma concentration and cerebral net exchange of NO (A, B) and CGRP (C, D). A positive value for exchange indicates net influx or uptake across the brain. *Different vs arterial sample site for given condition ($P<0.05$). †Different vs normoxia for given sample site ($P<0.05$).

ache,[17] and antagonism with BIBN 4096 BS provides effective prophylaxis.[18]

However, these findings have not been consistently reproduced caused, in part, by weak experimental design using historic control subjects and technical deficiencies associated with the accurate measurement of CGRP. Using an intrapatient comparative design and a more sensitive radioimmunoassay, a recent study failed to confirm any difference in CGRP levels from cubital and external jugular venous blood of patients during and outside of migraine attack without aura.[11]

This agrees with our current findings that adopted a similar experimental approach specifically designed to minimize the considerable interindividual variation associated with resting levels of CGRP. Incorporation of a homogenous group of apparently healthy males in the resting state excluded the potential confounding influence and interpretive complications associated with differences in sex, age, arterial hypertension, pregnancy, contraceptive pill use, and exercise on resting CGRP levels.[11] Thus, we remain confident that the lack of CGRP output across the brain reflects the true physiological response to hypoxia and is not an artifact caused by methodological limitations.

Table 2. Cephalalgia

Condition	Normoxia	Hypoxia
LL (points)	0±0	3±2*
ESQ-C (AU)	0.000±0.000	0.803±0.674*
VAS (mm)	0±0	29±23*

LL indicates Lake Louise; ESQ-C, Environmental Symptoms Questionnaire-Cerebral Symptoms; VAS, Visual Analogue Scale scores.

*Different vs normoxia ($P<0.05$).

However, because the half-life of CGRP has been estimated at only a few minutes,[19] we cannot exclude acute release from trigeminal perivascular nerve fibers, the site of migraine nociception, during the initial early phase of hypoxia. It is conceivable that the decreased arterial delivery of $NO_2^{\bullet}$ may reflect an increase in the systemic rate of $NO_2^{\bullet}$ reduction by deoxyhemoglobin to increase the intravascular pool of bioavailable NO[5] ($Hb\text{-}Fe^{2+}+NO_2^{\bullet}+H^+ \rightarrow Hb\text{-}Fe^{3+}+NO+OH^-$). The increase in NO may not only serve as a countermeasure to effect cerebral vasodilatation to preserve oxidative metabolism in the face of prevailing hypoxemia but may have also stimulated focal CGRP synthesis and release resulting in neurogenic vasodilatation, inflammation, hyperalgesia, and allodynia.[20] Alternatively, the blood–brain barrier, which we have shown to remain mostly intact during hypoxia,[13] may have also prevented intrathecally formed CGRP from entering the extracranial circulation in sufficient amounts to permit molecular detection.

However, what is apparent from the current findings is that the lack of CGRP exchange at 9 hours excludes sustained steady-state release of CGRP as the primary cause of AMS. Because accumulating evidence suggests that CGRP is considered a biological marker of peripheral trigeminal vascular system activation,[6] our findings suggest that alternative mechanisms are likely responsible for the high-altitude headache observed in AMS.

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Disclosure

None.

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