CALL FOR PAPERS | Oxygen as a Regulator of Biological Systems

Hypoxemia, oxygen content, and the regulation of cerebral blood flow

Ryan L. Hoiland,1 Anthony R. Bain,1 Mathew G. Rieger,1 Damian M Bailey,2 and Philip N. Ainslie1,2
1Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia-Okanagan Campus, Kelowna, British Columbia, Canada; and 2Neurovascular Research Laboratory, Research Institute of Science and Health, University of South Wales, Glamorgan, United Kingdom

Submitted 16 June 2015; accepted in final form 30 November 2015

Hoiland RL, Bain AR, Rieger MG, Bailey DM, Ainslie PN. Hypoxemia, oxygen content, and the regulation of cerebral blood flow. Am J Physiol Regul Integr Comp Physiol 310: R398–R413, 2016. First published December 16, 2015; doi:10.1152/ajpregu.00270.2015.—This review highlights the influence of oxygen (O2) availability on cerebral blood flow (CBF). Evidence for reductions in O2 content (CaO2) rather than arterial O2 tension (PaO2) as the chief regulator of cerebral vasodilation, with deoxyhemoglobin as the primary O2 sensor and upstream effector, is discussed. We review in vitro and in vivo data to summarize the molecular mechanisms underpinning CBF responses during changes in CaO2. We surmise that 1) during hypoxic hypoxia in healthy humans (e.g., conditions of acute and chronic exposure to normobaric and hypobaric hypoxia), elevations in CBF compensate for reductions in CaO2 and thus maintain cerebral O2 delivery; 2) evidence from studies implementing iso- and hypervolumic hemodilution, anemia, and polycythemia indicate that CaO2 has an independent influence on CBF; however, the increase in CBF does not fully compensate for the lower CaO2 during hemodilution, and delivery is reduced; and 3) the mechanisms underpinning CBF regulation during changes in O2 content are multifactorial, involving deoxyhemoglobin-mediated release of nitric oxide metabolites and ATP, deoxyhemoglobin nitrite reductase activity, and the downstream interplay of several vasoactive factors including adenosine and epoxyeicosatrienoic acids. The emerging picture supports the role of deoxyhemoglobin (associated with changes in CaO2) as the primary biological regulator of CBF. The mechanisms for vasodilation therefore appear more robust during hypoxic hypoxia than during changes in CaO2 via hemodilution. Clinical implications (e.g., disorders associated with anemia and polycythemia) and future study directions are considered.

cerebral blood flow; cerebral oxygen delivery; hypoxia; nitric oxide; adenosine triphosphate

Relative to other species, the human brain has evolved to be approximately three times larger than expected for body size due to increases in the frontal lobe, temporal lobe, and cerebellum (164). The increase in brain volume occurred during the early evolution of the genus Homo and was especially pronounced in Homo erectus due to selection pressures associated with greater social complexity, enhanced ecological demands on cognition, and increased physical activity (76, 154, 170). As a consequence, the brain has evolved into a highly oxidative organ accounting for a disproportionate 20% of the basal oxygen budget which is 10 times higher than what would be expected from its weight (153). The ability to process large amounts of O2 over a relatively small tissue mass is required to support the high rate of ATP production to maintain an electrically active state for the continual transmission of neuronal signals (reviewed in 7). However, this obligatory high rate of cerebral O2 consumption is associated with a commensurately high “vulnerability for failure.” In light of this vulnerability, adequate delivery of O2 to the brain via precise regulation of cerebral blood flow (CBF) is therefore vital to maintaining optimal function and avoid cellular damage and/or death. Describing the influence of oxygen (O2) availability on CBF and brain metabolism is an essential step toward a better understanding of brain energy homeostasis and associated clinical implications.

An appropriate and commonly employed model to investigate the acute and chronic cerebrovascular effects of reduced O2 availability involves exposure to normobaric hypoxia or ascent to high altitude (HA; over 3,000 m above sea level). It has been well documented that CBF increases in response to the severity of hypoxic stimuli in humans via cerebral vasodilation (2–5, 166, 199, 202). These compensatory increases in CBF upon exposure to normo- and hypobaric (sea level and
HA, respectively) hypoxia are adequate to maintain cerebral oxygen delivery (CDO2, reviewed in Ref. 5). The mechanisms underlying the influence of hypoxia upon CBF are complex and involve interactions of many physiological, metabolic, and biochemical processes. For example, potential mechanisms of cerebrovascular dilation likely change depending on the magnitude and duration of exposure to hypoxia, the degree of acid-base adjustment, intrinsic cerebral reactivity to changes in O2, CO2, and pH, as well as release of local vasoactive factors [e.g., nitric oxide (127) and adenosine (22, 135) to name but a few].

The partial pressure of arterial oxygen (PaO2) dissolved in the plasma contributes little to the total content of arterial oxygen (CaO2; Fig. 1); however, because the partial pressure gradient between arterial blood and tissue facilitates diffusion of O2 into the cell, PaO2 is often presumed to be the cerebral vascular stimulus during hypoxia. Yet, blood flow to contracting skeletal muscles is regulated by CaO2, not PaO2 (61), with deoxyhemoglobin (deoxyHb) being both the primary O2 sensor and upstream response effector; there are data in humans indicating the same might be true for CBF regulation. Moreover, in clinical and environmental (HA) conditions where CaO2 is elevated, there is evidence that CBF is reduced (84, 128).

Herein, for both physiological and to a lesser extent pathophysiological settings, we review the current knowledge of CBF regulation with changes in PaO2 and/or CaO2. Emerging evidence suggests that deoxyhemoglobin is the primary biological regulator of CBF, and therefore consequently CDO2 and ultimately brain tissue oxygenation, during changes in CaO2 originating from alterations in O2 tension (i.e., hypoxemic hypoxia), hemodilution, and anemia. First, we provide an overview of how CBF is regulated under acute (seconds to hours), chronic (days to years), and lifetime conditions of hypoxia, and examine the evidence supporting the hypothesis that deoxyhemoglobin (deoxyHb) is the primary biochemical processes. For example, potential mechanisms of cerebrovascular dilation likely change depending on the magnitude and duration of exposure to hypoxia, the degree of acid-base adjustment, intrinsic cerebral reactivity to changes in O2, CO2, and pH, as well as release of local vasoactive factors [e.g., nitric oxide (127) and adenosine (22, 135) to name but a few].

The partial pressure of arterial oxygen (PaO2) dissolved in the plasma contributes little to the total content of arterial oxygen (CaO2; Fig. 1); however, because the partial pressure gradient between arterial blood and tissue facilitates diffusion of O2 into the cell, PaO2 is often presumed to be the cerebral vascular stimulus during hypoxia. Yet, blood flow to contracting skeletal muscles is regulated by CaO2, not PaO2 (61), with deoxyhemoglobin (deoxyHb) being both the primary O2 sensor and upstream response effector; there are data in humans indicating the same might be true for CBF regulation. Moreover, in clinical and environmental (HA) conditions where CaO2 is elevated, there is evidence that CBF is reduced (84, 128).

Herein, for both physiological and to a lesser extent pathophysiological settings, we review the current knowledge of CBF regulation with changes in PaO2 and/or CaO2. Emerging evidence suggests that deoxyhemoglobin is the primary biological regulator of CBF, and therefore consequently CDO2 and ultimately brain tissue oxygenation, during changes in CaO2 originating from alterations in O2 tension (i.e., hypoxemic hypoxia), hemodilution, and anemia. First, we provide an overview of how CBF is regulated under acute (seconds to hours), chronic (days to years), and lifetime conditions of hypoxia, and examine the evidence supporting the hypothesis that deoxyhemoglobin (deoxyHb) is the primary biochemical processes. For example, potential mechanisms of cerebrovascular dilation likely change depending on the magnitude and duration of exposure to hypoxia, the degree of acid-base adjustment, intrinsic cerebral reactivity to changes in O2, CO2, and pH, as well as release of local vasoactive factors [e.g., nitric oxide (127) and adenosine (22, 135) to name but a few].
cerebral (e.g., MCA) (88, 201, 203) and extracranial cerebral vessels (e.g., internal carotid artery and vertebral artery) (116). This dilation has been observed when $\text{SaO}_2$ falls to $\sim 80\%$ for both the large intracranial (88) and extracranial (116) cerebral vessels.

Although the underlying mechanisms have not been fully elucidated in awake humans (see SIGNALING PATHWAYS IN THE REGULATION OF CBF DURING HYPOXIA), upon reanalysis of the key studies to date (see Fig. 2), the elevation in CBF in response to acute hypoxemic hypoxia seems to be entirely appropriate to maintain CDO2. When CDO2 is maintained, both cerebral oxygen extraction (%) and CMRO2 are also unchanged (4). However, if CDO2 were to become reduced, then oxygen extraction has the capacity to almost double to compensate. The CDO2 and cerebral oxygen extraction fraction can be calculated as per Eqs. 1 and 2:

$$\text{CDO}_2 (\text{ml/min}) = \text{gCBF} (\text{ml/min}) \cdot \text{CaO}_2 (\text{ml/dl}) / 100$$

$$\text{Cerebral O}_2 \text{ extraction } (%) = \left( \frac{(\text{CaO}_2 - \text{CjvO}_2)}{\text{CaO}_2} \right) \cdot 100$$

where CDO2 represents cerebral oxygen delivery, gCBF represents global cerebral blood flow, CaO2 represents the arterial content of oxygen, and CjvO2 represents the jugular venous content of oxygen.

While global CDO2 is maintained during hypoxemic hypoxia, there appears to be a moderately heterogeneous response throughout the brain, related to regional disparities in CBF and hypoxic reactivity (Fig. 3) and an overall drop in tissue PO2 (as inferred from jugular venous PO2) (4). These disparities in the maintenance of CDO2, and thus specific regional variations in tissue PO2, in concert with selective vulnerability of specific regions (i.e., selective neuronal vulnerability) to hypoxia (149) are likely implicated in cerebral dysfunction (e.g., reduced cognitive capacity). Additionally, independent of CDO2, tissue PO2 levels can directly regulate neuronal ion channel function (98) and alter neurotransmitter production (e.g., glutamate, serotonin, acetylcholine) due to a low $K_m$ for oxygen (60, 65).

Fig. 2. Cerebral blood flow (CBF) and oxygen delivery (CDO2) during acute hypoxemic hypoxia in humans. Data taken from five studies during hypoxemic hypoxia with concurrent measures of CBF and arterial blood gases. As exceptions we used data from Refs. 75 and 169, where Eq. 3 was used to calculate CaO2 with $\text{SaO}_2$ estimated using the Severinghaus equation (167). Data from 55 healthy subjects are depicted; i.e., $n = 7$ (108), ten (4), six (169), nine (29), ten (199), nine (200), and four (75). The mean lines for both CBF and CDO2 have been calculated as the linear slope from the mean data of each study weighted for sample size. All studies were conducted under isocapnic conditions except for Ref. 108, where $\text{PaCO}_2$ was reduced by 4 mmHg during the hypoxic exposure.

Chronic hypoxemic hypoxia (days to years): evidence from studies at high altitude. The influence of CaO2 on CBF is contingent on the balance between the degree and duration of hypoxia and ensuing hypocapnia. In turn, the extent to which the cerebrovasculature responds to HA is dependent upon four key integrated reflexes: 1) hypoxic cerebral vasodilation; 2) hypocapnic cerebral vasconstriction; 3) the hypoxic ventilatory response; and 4) the hypercapnic ventilatory response (reviewed in detail elsewhere; 5). Indeed, because pH and CaO2 change throughout the acclimatization process to hypoxia (i.e., metabolic compensation for the respiratory alkalosis, which returns pH towards baseline, and progressive increases CaO2,
from hemoconcentration), the CBF response to hypoxia will follow accordingly.

At least eight studies have measured CBF during acclimatization to HA (≥3,400 m) using a variety of techniques (e.g., Kety Schmidt, $^{133}$Xe, vascular ultrasound, transcranial, and transcranial color-coded Doppler; for review of measurement techniques, see 171, 185) to identify consistent increases in CBF following exposure to HA; however, the degree of hypoxia and duration of time at altitude is inconsistent and variable (11, 79, 96, 119, 158, 166, 175, 201). What is noteworthy, as illustrated in Fig. 4, is that the ~20–60% increase in CBF in each of the cited studies is closely matched to the reduction in $\text{CaO}_2$ in a reciprocal manner; CDO$_2$ is therefore well maintained across acclimatization.

Considering the importance of PaCO$_2$ on the cerebrovascular response to normobaric (29, 139, 199) and hypobaric hypoxia (200), the elevated CBF during initial exposure to HA, at first glance, appears paradoxical and variable (166). However, it is well established that individual variability in hypoxic and hypercapnic ventilatory sensitivities influences the onset of ventilatory acclimatization (i.e., degree of increased PaO$_2$ and decreased PaCO$_2$) (37). A recent study demonstrated that the onset of ventilatory acclimatization combined with metabolic compensation of the respiratory alkalosis over time results in a normalization of the CBF response despite the prevailing hypocapnia due to metabolic compensation of the respiratory alkalosis (200).

Another important factor that has been largely ignored in the regulation of CBF at HA is changes in $\text{CaO}_2$ and blood viscosity. As outlined in Fig. 4, $\text{CaO}_2$ is progressively increased after approximately the first week at HA due to ventilatory acclimatization, hemoconcentration, and acid-base changes. Hematocrit (Hct) is increased by 10–15% over the first few weeks at altitude (119) and therefore, due to the consequent elevation in $\text{CaO}_2$, also tends to lower CBF. Acute changes in blood viscosity may also affect endothelial functioning via changes in shear stress, and research has indeed shown that the cerebral vasculature exhibits a degree of autoregulation in response to both acute increases and decreases in plasma viscosity (131). The magnitude and direction of the change in viscosity may therefore affect the capacity of the blood vessel to respond to further changes in $\text{CaO}_2$. Alternatively, in conditions where viscosity is chronically increased (e.g., chronic mountain sickness, sickle cell disease), endothelial functioning is often blunted as increases in shear stress are offset by secondary complications such as heightened sympathetics and systemic inflammation (8).

**Chronic adaptation to high altitude.** Although hypoxia is a major physiological stress, several human populations have survived for millennia at HA, suggesting they have adapted to hypoxic conditions. Three successful patterns of human adaptation to high-altitude hypoxia have been documented (14): Andean (i.e., “classic adaptation,” erythrocytosis with arterial hypoxemia) (15); Tibetan (i.e., marginally elevated venous hemoglobin concentration with arterial hypoxemia) (14); and the recently identified Ethiopian—Amhara highlanders living at ~3,500 m—pattern [i.e., normal venous hemoglobin concentration and arterial oxygen saturation within the normal range of sea level populations (14)]. Of note, the Amhara pattern of adaptation exhibits higher O$_2$ saturation and less erythrocytosis than their Omotic Ethiopian counterparts (171).

Early studies have reported that native Andeans living at HA (~4,200 m) have 20% lower CBF values compared with sea-level natives (reviewed in 5). The main mechanism underpinning the ~20% lower CBF of high-altitude residents is the reported elevation in Hct and consequently increased $\text{CaO}_2$. These conclusions are largely based on the inverse relationship between $\text{CaO}_2$ and CBF that has been demonstrated with carbon monoxide exposure or hemodilution (122; see ARTERIAL OXYGEN CONTENT AND CEREBRAL BLOOD FLOW; HYPOXIC HYPERTENSION VS. HEMODILUTION). The lower CBF in high-altitude residents may also be attributed to the passive changes in blood viscosity associated with increased Hct (128) and active cerebral vasoconstriction (155, 156). For example, the cerebral arteriovenous oxygen content difference is approximately propor-

---

**Fig. 4.** Cerebral blood flow (CBF) and oxygen delivery (CDO$_2$) at high altitude. Percent change in cerebral blood flow ($\Delta$%CBF) during acclimatization (>4 days above 3,400 m) in the eight studies at various altitudes reviewed in Ref. 5 (11, 79, 96, 119, 158, 166, 175, 201), and one recent investigation following 5 days at 4,350 m (158). As depicted, CDO$_2$ is maintained at high-altitude due to the compensatory increase in CBF. As all studies were conducted during hypobaric hypoxia, PaCO$_2$ was not controlled. TCCD, transcranial color-coded Doppler; gCBF, global cerebral blood flow. [Adapted from Ainslie and Subudhi (5) with permission. Copyright Mary Ann Liebert, Inc.]
tional to the Hct level in high-altitude natives (128). However, via theoretical corrections in CBF for the elevations in Hct, it is calculated that CBF is still ~5% lower in high-altitude Andean natives (91). Whether the reduction in CBF in Andean high-altitude residents is a function of increased blood viscosity, increased arterial-venous oxygen content difference, or augmented oxidative-nitrosative stress (8), has yet to be resolved. Nevertheless, it appears, at least at sea level, that blood viscosity does not affect CBF and that the observed reductions are simply due to the corresponding elevation in CaO2 (see ARTERIAL OXYGEN CONTENT AND CEREBRAL BLOOD FLOW: HYPOXEMIC HYPONXIA VS. HEMODILUTION). Therefore, increased CaO2 is likely the primary factor governing the reduction in CBF noted in high-altitude natives.

Although CBF in Tibetan high-altitude residents at ~4,200 m seems 5–10% lower than lowlanders, these data are limited and based on blood velocity indices of the middle cerebral artery (MCA) (92–94). Jansen and Basnyat (91) contended that velocities in the internal carotid artery and MCA were 11.7% and 3.4% (mean 6.2%) higher, respectively, compared with lowlanders, yet still within the range of expected variation at sea level reported by others (162, 163). Also, Hct and CaO2, in Tibetan (Sherpa) high-altitude residents (200) are slightly increased compared with sea-level (by roughly 10%) but comparable to well-acclimatized lowlanders (52, 56, 119, 152). Despite the need for experimental evidence, an increased nitric oxide availability in Himalayans has been speculated to explain differences in CBF between Tibetan and Andean populations (13, 44). Likewise, although the cerebral circulation of Ethiopian HA dwellers seems to be insensitive to hypoxia and may represent a positive adaptation (unlike Andean HA dwellers), this is also solely based on MCA velocity (28). Since the MCA has been demonstrated to dilate in hypoxia (88, 201, 203), past indices of CBF at altitude based on MCA velocity need to be interpreted with caution.

ARTERIAL OXYGEN CONTENT AND CEREBRAL BLOOD FLOW: HYPOXEMIC HYPONXIA VS. HEMODILUTION

The magnitude of the CBF response is proportional to the severity of hypoxic stimuli and is appropriate to maintain CDO2 and hence offset the hypoxemic hypoxia-induced reductions in CaO2 (2–5, 166, 199, 202). However, an important prevailing question in human cerebrovascular physiology is whether the primary regulator of CBF is the total amount of O2 (i.e., CaO2) or the O2 dissolved in plasma (i.e., PaO2). The amount of oxygen bound to hemoglobin and PaO2 determine CaO2, as per Eq. 3:

\[
CaO2 (ml/dl) = [Hb] \times 1.36 \times (\%SaO2/100) + 0.003 \times PaO2
\]

where [Hb] is the arterial hemoglobin concentration, 1.36 is the affinity for O2 to hemoglobin, and 0.003 is the solubility of O2 dissolved in blood.

Although PaO2 (dissolved O2) only minimally contributes to the total CaO2 when breathing room air, because it facilitates diffusion of oxygen into the cell, PaO2 is often presumed to be the cerebral vascular stimulus. Yet, as previously discussed, CBF does not decrease until a PaO2 of ~50 mmHg, the point whereby the reduction in CaO2 (and SaO2) accelerates with further decreases in PaO2 (Fig. 1). Reductions in CaO2 with maintained PaO2, resulting from either carbon monoxide exposure (142), acute or chronic anemia (24, 67), and hemodilution (142), all increase CBF. Moreover, CBF varies inversely with Hct in many species in both acute (e.g., acute anemia) and chronic experimental conditions (e.g., erythropoiesis/polycythemia). While all three of these experimental paradigms (anemia, hemodilution, and carbon monoxide exposure) highlight the coupling of CBF to CaO2, it is important to consider their biological differences. For example, carbon monoxide is a cerebral vasodilator independent of inhibiting the formation of oxyhemoglobin (113), and elicits a greater increase in CBF for a given reduction in CaO2 than hemodilution (142). Therefore, comparing the differences in vasodilation mediated by hypoxemic hypoxia (i.e., ↓ PaO2) and isovolumic hemodilution (simulated acute anemic hypoxia) represents a more robust model than using carbon monoxide. As the underlying mechanism(s) remain largely theoretical, it has been speculated that 1) the hemorheologic consequences of reductions in blood viscosity (which is derived from plasma viscosity, Hct, and mechanical properties of red blood cells) mediate the increases in CBF noted during hemodilution (e.g., 177, 178); and 2) reductions in CaO2 elicit vasodilatory responses to maintain CDO2 (e.g., 24, 26). The following sections highlight the relationship between CaO2 and CBF in the presence and absence of changes in PaO2. The potential governing mechanisms are discussed.

The majority of animal studies have indicated that viscosity is an important regulator of CBF during hemodilution (80, 109, 183); however, this is not a universal finding (195). Moreover, studies in humans provide evidence against an appreciable role of viscosity. Indeed, despite the initial few human studies supporting that viscosity is a primary regulator of CBF during changes in Hct (64, 83, 84, 178), the potential implications of changes in CaO2, and its consequent effects on CDO2 were ignored. To partition the influence of blood viscosity independently of Hct and CaO2, Humphrey et al. (82) investigated the difference in CBF between paraproteinemic (patients with high plasma protein concentration and elevated plasma viscosity) and nonparaproteinemic patients (82). However, both Hct and PaCO2 differed between groups. If the average CBF difference between paraproteinemic and nonparaproteinemic groups are corrected for the differences in Hct (assumed to be indicative of differences in CaO2), and PaCO2 (using the linear slope presented in Fig. 5 and the known ~7% increase in volumetric CBF per mmHg change in PaCO2 above eupneic levels (108, 199), respectively), the observed difference in CBF is abolished. The limitations of this study were addressed in a later study by Brown and Marshall (26); here, changes in viscosity failed to alter CBF when CaO2 and PaCO2 remained constant. In turn, the authors suggested that independently viscosity is an insignificant factor in CBF regulation after accounting for changes in CaO2 (24). This finding has been replicated in other clinical populations possessing intact cerebrovascular function and normal Hct during isovolumic conditions (25) as well as during hemodilution-mediated hypoxia in animals using high- and low-viscosity replacement fluids (195). Therefore, it seems the impact of viscosity may be negligible in the hypoxic brain at rest, and relative to hypoxic CBF reactivity where the vascular bed becomes vasodilated. Nevertheless, theoretically, Poiseuille’s law demonstrates that changes in viscosity will have pronounced effects on flow, as per Eq. 4:
Analysis of 20 studies shows that CaO2 is inversely related to CBF increase to the cerebral circulation relative to other organs. The greater blood flow during hemodilution, one might expect that blood flow to all organs if viscosity was the primary factor regulating CBF during hemodilution, and hypoxia in humans (24). Moreover, negative findings regarding an effect of viscosity on CBF and those that define Poiseuille’s law likely explains the disparity between physiological conditions (turbulent flow and non-Newtonian fluid) and those that define Poiseuille’s law likely explains the negative findings regarding an effect of viscosity on CBF during hemodilution, and hypoxia in humans (24). Moreover, if viscosity was the primary factor regulating CBF during hemodilution, one might expect that blood flow to all organs would increase to the same magnitude. The greater blood flow increase to the cerebral circulation relative to other organs indicates a selective active regulation (21). Collectively, analysis of 20 studies shows that CaO2, is inversely related to CBF (Fig. 6).

The CBF response during hemodilution and hypoxic hypoxia is tightly coupled to reductions in CaO2 [e.g., for isovolumic hemodilution (33, 47, 73, 130, 211) and for hypoxic hypoxia (4, 29, 108, 199]; see Figs. 2 and 5]. Thus, in both otherwise healthy individuals and those with pathology (e.g., polycythemia, paraproteinemia), with normal (26) or high Hct (84), isovolumic hemodilution leads to an increase in CBF. Of note, the slope increase in CBF during isovolumic hemodilution is markedly (3-fold) reduced compared with during hypoxic hypoxia (Fig. 7). The mean slope increase in CBF during isovolumic hemodilution is 0.66% CBF/-%CaO2, whereas the mean slope increase in CBF during hypoxic hypoxia is 1.85% CBF/-%CaO2 (see Figs. 2 and 5 legends for explanation of calculations). The blunted cerebrovascular response to hemodilution compared with hypoxic hypoxia results in a lowered CDO2 (Fig. 7). When assessing the long-term influence of CaO2 from altered hemoglobin mass in anemia and polycythemia, it is apparent that CDO2 is not impaired (Fig. 6). Indeed, patients with anemia possess a relatively high CBF that maintains CDO2 despite reduced CaO2, and the reduction in CBF that is typical of polycythemic patients is not large enough to reduce CDO2, which appears to remain normal in these patients.

In the study by Daif et al. (33), where jugular venous samples were collected, an approximate 10% reduction in CMRO2 during hemodilution was estimated (33). This is in contrast to the multiple studies assessing CMRO2 during hypoxic hypoxia, where a 35% reduction in CaO2 does not result in an altered CMRO2 (4, 29, 108). Whether the reduction in estimated CMRO2 calculated by Daif et al. (33) is due to the impaired CDO2 following hemodilution, or simply confounded by the concurrent anesthesia, is unknown (140). The remainder of human (73, 142) and animal (181) data indicate that CMRO2 remains unchanged during hemodilution despite a CaO2 reduction of 30%, due to a compensatory increase in cerebral O2 extraction (73, 181).

While within-subject manipulation of CaO2 through various methods (e.g., hemodilution vs. hypoxic hypoxia) to assess cerebral vascular regulation to hypoxia has yet to be done in healthy human subjects, we speculate that the supposed differential response between hemodilution and hypoxic hypoxia (that is, greater CBF increase with hypoxic hypoxia compared with hemodilution) is due to the fundamental mechanisms governing hypoxic cerebral vasodilation. The mechanisms governing cerebrovascular vasodilation to hypoxia are now discussed, with speculation as to how they contribute to the response characteristics of hemodilution and hypoxic hypoxia-mediated increases in CBF.

**Signalizing Pathways in the Regulation of CBF During Hypoxia**

Although the cerebrovascular responses to acute and chronic hypoxia during both normobaria and hypobaria have been well characterized in humans, there remains a paucity of data related to the cellular mechanisms that govern it. Ultimately, it seems that hemoglobin in the erythrocyte functions as the primary O2 sensor and upstream response effector governing the regulation of vascular tone in hypoxia. Three principal mechanisms have been proposed for erythrocyte-dependent hypoxic vasodilation and include: 1) ATP release and subsequent activation of endothelial nitric oxide synthase (eNOS); 2) nitrite reduction to NO by deoxyhemoglobin, and 3) S-nitrosohemoglobin (SNOHb)-mediated bioactivity. Ultimately, it seems that hypoxic vasodilation hinges upon deoxyhemoglobin-mediated release of nitric oxide (i.e., S-nitrosothiols) and ATP, as well as NO reductase activity, which are all dependent on transition of hemoglobin from the relaxed (R) state to tense...
These processes have direct vasomotor effects and influence release and/or formation of specific signaling molecules that are integral to the vasodilatory response. Candidate mechanisms (see Fig. 8) responsible for vasodilation downstream of deoxyhemoglobin include nitric oxide, adenosine, prostaglandins, and expoxyeicosatrienoic acids.

The site of hemoglobin deoxygenation (R → T allosteric shift) is not exclusive to the capillaries as over 66% of blood oxygen can be removed in the upstream cerebral arterial circulation prior to reaching the capillaries (46, 185). This highlights that erythrocyte release of ATP, SNO, and nitrite reductase activity can mediate dilation throughout the cerebral arterial tree. Moreover, these signals can be propagated (40, 41, 49, 102), and therefore, likely impact further upstream resistance vessels that only see oxygenated blood. Indeed, hypoxia has been shown to dilate the MCA (88, 201, 203), internal carotid artery (116), and vertebral artery (116) in humans.

**Deoxyhemoglobin-Mediated Signal Transduction Pathways**

*S*-nitrosohemoglobin release from erythrocytes during hypoxia. Transport to and release of NO in the microvasculature are achieved in part through *S*-nitrosylation of hemoglobin. Formation of *S*-nitrosohemoglobin (SNO-Hb) in the lung occurs due to covalent bonding of NO or *S*-nitrosothiol (SNO) with the β-chain of β93 cysteine (Cys β93) of the Hb molecule (97). *S*-nitrosylation of Hb to form *S*-nitrosohemoglobin occurs most effectively in the R-state (i.e., in the lungs; Ref. 84) and allows for transport of vasoactive NO to the cerebral vasculature, where SNO is released upon deoxygenation of hemoglobin and transition to the T-state (174). When released, *S*-nitrosothiol provides the chemical stability required for NO to reach the endothelium as free NO would be scavenged too quickly to elicit vasodilation (186). The presence of a negative arterial to jugular venous SNO gradient indicates its transport and release in the rat brain (97). Moreover, it seems that the oxygenation state of cerebral tissue Po2 affects the vasodilatory influence of SNO (174), and regulates its role across physiological oxygen gradients.

**Nitrite reduction via hemoglobin.** Nitrite (NO\(_2^-\)) acts as a storage pool for NO. There is accumulating evidence that its reduction by hemoglobin during hypoxia is an integral component of NO-mediated vasodilation (30, 43). The time course for NO\(_2^-\) reduction by hemoglobin is on the order of seconds to

---

---
minutes (43), and thus likely contributes primarily to steady-state CBF vs. that of the initial upslope upon hypoxia exposure. In humans, after 9 h of passive exposure to normobaric hypoxia, the arterial delivery of NO$_2^-$ to the brain was reduced indicating that it was either reduced by deoxyhemoglobin to indicate that it was either reduced by deoxyhemoglobin to below.

ponents regulates erythrocyte-mediated ATP release and may affect ATP release from erythrocytes during hypoxia.

The liberation of ATP from deoxyhemoglobin is positively related to temperature (104).

Temperature and ATP release. The liberation of ATP from deoxyhemoglobin is positively related to temperature (104). However, acute hypoxia alone at rest does not independently change core temperature; therefore the role of temperature on the CBF response to hypoxia alone is likely negligible. Nevertheless, any increase in CBF will increase the local cerebral heat loss (10, 70, 137). Theoretically, assuming a constant cerebral metabolic rate and arterial temperature, a 50% increase in CBF (e.g., with hypoxia) would increase heat removal from the brain by 50%. An extra 0.33 J·g$^{-1}$·min$^{-1}$ (209) of heat dissipated would result in a cerebral temperature reduction of $\sim$0.09°C/min (assuming a tissue specific heat capacity of 3.6 J·g$^{-1}$·°C$^{-1}$). However, a notable reduction in cerebral temperature would result in a reduction of the cerebral metabolic rate (10), and current evidence indicates that the cerebral metabolic rate of oxygen during isocapnic hypoxemic hypoxia is not reduced (4). In turn, it is unlikely that the liberation of ATP from deoxyhemoglobin is altered by potential changes in cerebral temperature with hypoxia.

Shear Stress and ATP Release. Shear stress and deformation of red blood cells facilitate the release of ATP from erythrocytes in vitro (173, 194). Increased blood velocity during hypoxia will lead to increased shear and thus lead to increased ATP release from erythrocytes. Shear is therefore likely an important factor mediating ATP release from red blood cells during hypoxia.

ATP. ATP is a potent cerebral vasodilator. Intraluminal ATP dilates the MCA of rats in vitro (32, 78, 212, 213), while in vivo intracarotid infusion of ATP increases pial vessel diameter in cats and global CBF in baboons (55). This ATP-mediated dilation is endothelium dependent (212, 213) through binding of P2Y$_2$ purinoceptors (40, 123), is capable of retrograde propagation (41, 102), and acts through initiating downstream signal cascades. The candidate downstream signals responsible for ATP-mediated changes in vascular tone include NO (212), adenosine (58), EETs (40, 101), and prostaglandins (114, 124). Collectively, these vasoactive factors increase K$^+$ channel conductance (77, 87), hyperpolarize the vascular smooth muscle (40, 102, 213), and/or decrease smooth muscle cell calcium sensitivity (1, 107). Evidence for an important role of electron transfer to ATP downstream of ATP binding to endothelial P2Y$_2$ receptors, which we propose as the initial step mediating the CBF response to hypoxia (see ATP liberation from erythrocytes during hypoxia) is discussed, with particular focus on data in humans.

Adenosine. Extravascular adenosine application leads to in vitro dilation of animal (41) and human (180) cerebral vessels and in vivo (17, 129, 193) dilation of animal cerebral vessels. This dilatory response is capable of retrograde propagation (102) and is mediated in part through increases in NO, inward rectifying potassium channel conductance (71), and increased cAMP levels (136, 161).

Hypoxia leads to an increase in the endogenous cerebral production of adenosine (17, 147, 204, 205). Production of adenosine in cerebral tissue is vital as negligible amounts of adenosine are thought to cross the blood-brain barrier and thus intravascular adenosine likely plays a limited role in CBF regulation during hypoxia (17). Administration of adenosine receptor antagonists (e.g., theophylline) abolishes the CBF increase to moderate hypoxia (i.e., PaO$_2$ = 40–50 mmHg), and all but one study in animals—albeit CBF was still moderately reduced (148)—indicate a reduced CBF increase to severe
Fig. 8. Putative pathways regulating cerebral blood flow during hypoxia. Increased temperature, erythrocyte deformation, and the conformational change concomitant to transition of oxy- to deoxyhemoglobin all signal erythrocyte mediated release of ATP (16, 49, 104, 173). Released ATP can then bind to the erythrocyte P2X<sub>7</sub> receptor in an autocrine fashion to induce erythrocyte mediated EET release (101), which will increase vascular smooth muscle cell K<sup>+</sup> channel conductance (39). The released ATP also binds endothelial P2Y<sub>1</sub> receptors to initiate a signal cascade involving NO and potentially PGs (212). Moreover, ATP will break down into AMP and subsequently adenosine (58) that will also exert a vasodilatory effect on vascular smooth muscle through binding adenosine A<sub>2A</sub> receptors (71, 103), increasing cAMP levels (136, 161) and also through increasing inward rectify potassium channel conductance (107). Cyclic nucleotides will also increase potassium channel conductance (20). Notionally, hypoxia increases guanylate cyclase activity and cGMP (145) as well as directly increase K<sup>+</sup> channel conductance (20). Cyclic nucleotides will also increase potassium channel conductance (172), with increased potassium efflux hyperpolarizing cells and reducing activity of voltage-gated Ca<sup>2+</sup> channels (134). Overall, ATP leads to vasodilation that can be conducted through gap junctions (41, 102). Green arrows represent activation of a downstream factor, and red arrows represent inhibition of downstream factors.

hypoxia (i.e., PaO<sub>2</sub> = 20–30 mmHg) by ~50% (50, 74, 129). This effect of attenuating CBF during hypoxia by adenosine receptor antagonism is reflected in reductions of pial arteriolar dilation to hypoxia subsequent to administration of adenosine deaminase (6) and theophylline (6, 146). However, this latter finding is not universal (66) and may depend on the severity of hypoxia. Adenosine A<sub>2A</sub> receptor knockout mice possess a finding is not universal (66) and may depend on the severity of hypoxia subsequent to administration of adenosine deaminase (71, 103). Increased temperature, erythrocyte deformation, and the conformational change concomitant to transition of oxy- to deoxyhemoglobin all signal erythrocyte mediated release of ATP (16, 49, 104, 173). Released ATP can then bind to the erythrocyte P2X<sub>7</sub> receptor in an autocrine fashion to induce erythrocyte mediated EET release (101), which will increase vascular smooth muscle cell K<sup>+</sup> channel conductance (39). The released ATP also binds endothelial P2Y<sub>1</sub> receptors to initiate a signal cascade involving NO and potentially PGs (212). Moreover, ATP will break down into AMP and subsequently adenosine (58) that will also exert a vasodilatory effect on vascular smooth muscle through binding adenosine A<sub>2A</sub> receptors (71, 103), increasing cAMP levels (136, 161) and also through increasing inward rectify potassium channel conductance (107). Cyclic nucleotides will also increase potassium channel conductance (20). Notionally, hypoxia increases guanylate cyclase activity and cGMP (145) as well as directly increase K<sup>+</sup> channel conductance (20). Cyclic nucleotides will also increase potassium channel conductance (172), with increased potassium efflux hyperpolarizing cells and reducing activity of voltage-gated Ca<sup>2+</sup> channels (134). Overall, ATP leads to vasodilation that can be conducted through gap junctions (41, 102). Green arrows represent activation of a downstream factor, and red arrows represent inhibition of downstream factors.

**Nitric oxide.** The potent vasodilatory and cardiovascular properties of nitric oxide are well documented (19), and likely extend into hypoxic CBF regulation. Nitric oxide induces vasodilation through increases in cyclic guanosine monophosphate (cGMP) (145) and directly mediated increases in K<sup>+</sup> channel conductance (20). Notionally, hypoxia increases neuronal nitric oxide synthase activity (12, 81, 160), releases NO from NO<sub>2</sub> (30) and SNO-Hb (117) (discussed in previous sections), and leads to endothelial NO release downstream of ATP signaling (212). In turn, NO is consistently involved with in vivo and in vitro animal cerebral hypoxic vasodilation (111, 143, 146, 176). In awake humans, intravenous infusion of NG-monomethyl-l-arginine (l-NMMA), a NOS inhibitor, reduces the hypoxic CBF response, as assessed via MRI (127). In contrast, Ide et al. (86) reported that NOS inhibition with l-NMMA does not influence the CBF response to acute hypoxia, as assessed by MCA velocity measures. The latter finding, however, is confounded by recent MRI and ultrasound evidence demonstrating that the MCA dilates in normobaric hypoxia (88, 203). As previously highlighted in Eq. 4, even the slightest changes in MCA diameter will have a profound effect on flow, due to their exponential relationship. Increases in shear stress via increased velocity through cerebral vessels during hypoxia likely provide an additional stimulus to upregulate endothelial NOS (42) and modulate NO signaling. Experiments implementing pharmacological blockade of NOS (e.g., via l-NMMA) do not however provide indication of the vasoactive influence of NO from Hb-SNO and NO<sub>2</sub> reduction and require parsimonious interpretation. While both animal and human data provide evidence for NO as an important regulator of the hypoxic CBF response, they relate only to NO derived locally from NOS and likely underestimate the true contribution of NO to hypoxic cerebral vasodilation.
**Prostaglandins.** Indomethacin (INDO), a nonsteroidal anti-inflammatory drug that nonselectively and reversibly inhibits cyclooxygenase activity, and consequent prostaglandin synthesis, has no effect on the CBF response to severe hypoxia ($\text{PaO}_2 = 25$ mmHg) in vivo in rats (159) and newborn pigs (31, 114), despite abolishing hypoxic dilation in vitro (57). Further evidence highlights that prostaglandins are not one of the endothelium-derived hyperpolarizing factors released downstream of ATP binding P2Y$_2$ receptors (40, 77). However, hypoxia increases cerebral 6-keto-PGF$_{1\alpha}$ (a stable prostacyclin metabolite), which is indicative of increased prostaglandin production (114, 124).

In humans, one study (75), but not all (53, 69), reports that INDO reduces MCA velocity reactivity to hypoxia. However, volumetric flow reactivity of the internal carotid artery during hypoxia is unaffected (75). In contrast, hypoxic reactivity seems to be reduced in the posterior circulation, assessed by vertebral artery blood flow, following cyclooxygenase inhibition with INDO (75). While these data indicate that cyclooxygenase may be selectively mediating hypoxic dilation in the posterior cerebral circulation, they are not conclusive. For example, INDO selectively reduces cerebral reactivity to hypercapnia (elevated arterial CO$_2$) whereas other cyclooxygenase inhibitors such as aspirin and naproxen have no effect (51, 122). These findings suggest INDO may influence CBF responses via a prostaglandin-independent mechanism. Indeed, INDO inhibits cAMP-dependent protein kinase activity (63, 105), which will directly impact smooth muscle tone (1, 107). More work is required to establish a role (if any) of prostaglandins in the cerebral hypoxic vasodilatory response in humans.

**Epoxyeicosatrienoic acids.** Epoxyeicosatrienoic acids dilate cerebral blood vessels (59) and are released from erythrocytes both spontaneously (100) and by ATP binding of P2X$_7$ receptors (101). Notably, this ATP binding also inhibits erythrocyte release of the vasoconstrictor 20-hydroxyeicosatrienoic acid (99, 101). During hypoxia, both inhibition of EET synthesis and EET antagonism markedly reduces the CBF response to hypoxia in rats (118) and newborn pigs (115). While epoxyeicosatrienoic acids may represent an endothelium-derived hyperpolarizing factor that is a relevant regulator of CBF during hypoxia, this has not been verified in humans.

**Cellular Regulation of Vascular Smooth Muscle Tone**

Fundamentally, there are two primary ways in which smooth muscle tone is regulated: 1) through changes in intracellular calcium concentration, and 2) through changes in vascular smooth muscle cell calcium sensitivity. Calcium entry into cells is governed largely by voltage-gated calcium channels. Hyperpolarization of the vascular smooth muscle cells by potassium efflux will downregulate calcium channel activity, making potassium channel conductance an important factor in the regulation of smooth muscle tone (134). Calcium sensitivity is dependent upon the phosphorylation (activation) of myosin, by myosin light chain kinase, which itself is dependent on cyclic nucleotide activity (cAMP and cGMP; 1).

**Potassium channels.** Increased conductance of potassium channels hyperpolarizes cells, decreases membrane potential and ultimately inhibits activity of voltage-gated calcium channels (54, 134). Several vasoactive factors affect potassium channel conductance during hypoxia. Adenosine, through binding of smooth muscle A$_2\alpha$ receptors, and vasodilator prostaglandins (e.g., PGI$_2$ and PGE$_2$) through binding of smooth muscle IP and EP receptors (35), increase intracellular cyclic nucleotides (71, 133), which results in increased potassium channel conductance. Moreover, EETs mediate cerebrovascular dilation through modulation of calcium-activated potassium channel conductance (59). In keeping, hypoxia reduces calcium influx in rabbit basilar arteries (144) and bovine middle cerebral arteries (188). The functional importance of potassium channels is further evidenced following K$_{ATP}$ channel blockade with glibenclamide, which reduces both pial vessel dilation (168) and the overall CBF response to hypoxia (118, 182). However, inhibition of potassium channels does not completely inhibit the CBF response to hypoxia, indicating that they are not the only regulator of CBF downstream of vasoactive agents (e.g., adenosine, NO, PGs, and EETs).

**Cyclic nucleotides.** Cyclic nucleotides (cAMP and cGMP) exert vasodilatory (184) effects primarily via two pathways; 1) by increasing conductance through potassium channels (172), and 2) by decreasing vascular smooth muscle cell calcium sensitivity. Cyclic nucleotides reduce smooth muscle cell calcium sensitivity by phosphorylating myosin light chain kinase, which leads to its deactivation, and a consequent reduced myosin-actin binding (1, 107).

**Mechanisms Leading to a Compromise in Cerebral O$_2$ Delivery During Hemodilution**

In contrast to during hypoxic hypoxia, data in humans (33, 47, 73, 130) and animals (181, 182) (Fig. 9) indicate that CDO$_2$ is impaired during reductions in CaO$_2$ via hemodilution. This may be due to a reduced signal transduction of processes originating from the erythrocyte, due to: 1) a reduction of total
hemoglobin levels during hemodilution compared with hypoxic hemoglobin, and 2) a reduction of deoxyhemoglobin produced from gas exchange with cerebral tissue during hemodilution compared with hypoxic hemoglobin (i.e., smaller percentage of deoxyhemoglobin). In humans, during hemodilution, jugular venous saturation is not appreciably reduced (<3% change) compared with baseline (33, 142), whereas during hypoxic hemoglobin jugular venous saturation is reduced progressively (up to ~20%) with increases in severity (4). The higher SaO2 during hemodilution would result in reduced deoxyhemoglobin-mediated ATP release, nitrite reductase activity, and NO release from SNO (34, 141, 197, 208).

Although the CBF response to a reduced CaO2 is impaired during hemodilution, it is not abolished; thus it seems that some regulatory mechanisms remain intact. Animal studies suggest that NO is a primary factor mediating the CBF response to hemodilution predominantly through upregulation of neuronal nitric oxide synthase (81, 125). The CBF response during hemodilution may also be blunted in part from the reduced blood viscosity, and therefore the reduced shear (106), which could hinder endothelium-mediated NO release (34, 141, 197, 208).

Clinical Implications and Future Directions

During or following major surgery (e.g., cardiac artery bypass, graft, transplant, etc.), patients often become acutely hemodiluted and are at risk of suffering acute neurological injury and long-term neurological impairment (132, 165). Indeed, evidence from animal models indicates that hemodilution reduces tissue oxygenation during cardiopulmonary bypass (45) and increases neuronal and mitochondrial injury (38). For example, in patients refusing blood transfusion during surgery (for religious reasons), preoperative [Hb] < 8 g/dl increased the risk of death 16-fold compared with those of higher [Hb] (27). During anesthesia, hemodilution leads to a reduction in CMRO2 (33), which may be indicative that CDO2 is not adequate to maintain metabolic homeostasis, as would normally occur during hypoxic hemoxia (4). Moreover, the occurrence of tissue hypoxia in traumatic brain injury patients with a tissue oxygenation probe was greater in patients with [Hb] of 7 g/dl vs. [Hb] of 10 g/dl (210). Whether the reduced vasomotor response and thus impaired maintenance of CDO2 during hemodilution is implicated in risk for neurological injury in humans during major surgeries requires further investigation (68). In addition to the implications for various surgical interventions, elucidation of these mechanistic CBF relationships is paramount to our comprehension of myriad pathologies associated with arterial hypoxemia (e.g., sleep apnea, chronic lung diseases, and heart failure) and/or alterations in blood viscosity or hemoglobin levels (e.g., anemic or polycythemic pathologies including von Hippel-Landau or Chuvash diseases). Uncovering the molecular basis of hypoxic adaptation in humans will both inform understanding of hematological and other adaptations involved in hypoxia tolerance and form the basis of novel methods for treating conditions of pathological brain hypoxia. Optimizing CDO2 via multi-modal imaging and/or molecular approaches may provide new insight into the treatment of many hypoxic, anemic, or polycythemic pathologies and that associated with cerebrovascular complications.

Conclusions

During hypoxic hemoxia at both sea level and high altitude in healthy humans, elevations in CBF are intimately matched to reductions in CaO2 to maintain CDO2. Studies employing hemodilution, and those of patients with anemia and polycythemia, support the notion that CaO2 has an independent influence on CBF; yet, in the majority of cases when CaO2 is reduced by hemodilution, CDO2 is compromised. The mechanisms regulating CBF during changes in CaO2 are multifactorial but primarily triggered by deoxygenation of hemoglobin (R → T allosteric shift) and consequent erythrocyte release of ATP and SNO, and deoxyhemoglobin nitrite reductase activity. Downstream of this initial process, additional chemical mediators include adenosine, nitric oxide, and epoxyeicosatrienoic acids. The relatively lower CBF increase with hemodilution compared with hypoxic hemoxia (due to ↓ [Hb] and higher jugular venous O2 saturation) provides strong evidence for the role of deoxyhemoglobin as the primary regulator of CBF in hypoxia. Although studies to date support the role of CaO2, as a biological regulator of CBF, due to the dependence of key regulatory mechanisms on the oxygenation state of hemoglobin, maintenance of O2 delivery via CBF is better established during hypoxic hemoxia, compared with hemodilution.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES

6. Armstrong WM. Role of nitric oxide, cyclic nucleotides, and the activation of ATP-sensitive K⁺ channels in the contribution of adenosine to


122. Matsumoto S, Sugimoto Y, Ushikubi F, Prostanoid receptors: structures, properties, functions. \( \text{Physiol Rev 79: 1193–1227, 1999.} \)


