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Gas Transport and Exchange: Interaction Between O_2 and CO_2 Exchange ☆

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Glossary

Arterial Adjective pertaining to blood that is flowing away from the heart; typically oxygenated blood.
Bohr effect Effect of the proton (H^+) concentration (pH) on the oxygen affinity of hemoglobin.
Carbonic anhydrase A zinc metalloenzyme that reversibly catalyzes the reaction between CO_2 and H_2O to form H^+ and HCO_3^-.
Equilibrium Pertaining to the situation when all forces acting are balanced by others resulting in a stable unchanging system.
Haldane effect Proton (H^+) and CO_2 binding to hemoglobin upon deoxygenation.
Hypercarbia High levels of carbon dioxide in water or air.
P_{50} The partial pressure of oxygen at which 50% of the hemoglobin molecules are oxygenated
Partial pressure The pressure that one gas would have if it alone occupied the same volume at the same temperature as the mixture.
Root effect Effect of the proton (H^+) concentration (pH) on the capacity of hemoglobin to be fully saturated with oxygen, even at extremely high oxygen partial pressures.
Venous Adjective pertaining to blood that is flowing back to the heart, typically after passing through tissues and unloading oxygen for metabolism.

Introduction

All animals produce approximately the same amount of CO_2 as O_2 consumed through the process of metabolism. The O_2 is taken up from the environment and delivered to the tissues by the blood, and the CO_2 is released from the tissues and transported by the blood for release back into the environment. Hemoglobin (Hb), which is encapsulated within the red blood cells (RBC), plays a vital role in both O_2 and CO_2 transport in the blood of all vertebrates (with the exception of some icefish, the only vertebrates lacking Hb). This article focuses on the nature of the interaction between O_2 and CO_2 at the level of Hb, a topic that has been well studied since the dual role of Hb was discovered in the early 1900s.

Oxygen uptake at the gas-exchange organ (referred to as the gills from this point forward, although the skin and various air-breathing organs can contribute to gas exchange in some fishes) also facilitates CO_2 removal through the Haldane effect. The process of Hb-oxygenation releases H^+ that combine with HCO_3^- to form CO_2, which diffuses into the environment. The CO_2 removal at the gills and the associated increase in blood pH results in an increase in Hb-O_2 affinity, which increases the driving force for O_2 diffusion across the gills, ultimately facilitating O_2 uptake via the Bohr effect. At the tissues, the reverse occurs. CO_2 diffusion into the blood creates an acidosis that facilitates O_2 delivery to the actively metabolizing tissues via the Bohr effect. Oxygen delivery facilitates CO_2 uptake by Hb, and thus CO_2 removal from the tissues via the Haldane effect. Thus, there is an intimate interaction between O_2 and CO_2 transport at both the gills and the tissues, which is facilitated by the Hb within the RBC and determined in part by the magnitude of the Bohr and Haldane effects.

Tremendous diversity in Hb characteristics exists within the fishes, making this group of animals particularly interesting for investigating the interaction between O_2 and CO_2 exchange. Some species possess very small, even nonexistent Bohr and Haldane effects with relatively high Hb-buffer values, substantially limiting the interaction between O_2 and CO_2 exchange. However, the majority of teleost species possess large Bohr and Haldane effects, collectively resulting in tightly coupled O_2 and CO_2 exchange. In addition, many teleost species also possess a Root effect associated with their Hb, where oxygen-carrying capacity of the Hb is reduced at low pH even at atmospheric oxygen tensions. Furthermore, many teleost fishes exhibit a nonlinear Bohr and Haldane effect over the region of the oxygen-equilibrium curve (OEC), which have further implications for the nature of the interaction between O_2 and CO_2 exchange.

Basis for the Interaction Between O_2 and CO_2: Bohr–Haldane Effect

The interaction between O_2 and CO_2 exchange is largely determined by the Bohr and Haldane effects, as discussed above; however, their respective magnitudes are important in determining the nature of the interaction. The Bohr effect describes how the affinity of

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Hb for O_2 is affected for a given change in the H^+ concentration (pH) of the blood. It is calculated as follows:

$$\text{Bohr coefficient} = -\Delta\log P_{50}/\Delta\text{pH} \quad [1]$$

where P_{50} refers to the partial pressure of O_2 (P_{O_2}) at which 50% of the Hb molecules are oxygenated.

The Haldane effect describes how the affinity of Hb for H^+ and CO_2 is affected by changes in Hb-O_2 saturation. It is calculated as follows:

$$\text{Haldane coefficient} = \Delta H^+ \quad [2]$$

where ΔH^+ refers to the moles of H^+ released per mole of O_2 bound to Hb.

Although the Bohr and Haldane effects are often discussed in terms of their respective roles relative to O_2 and CO_2 dynamics at the level of the Hb, they are actually mirror images of the same phenomenon. While the Bohr effect describes changes in Hb-O_2 affinity that arise from a change in H^+ concentration, the Haldane effect describes the changes in Hb–H^+ affinity that arise from a change in P_{O_2}, and therefore Hb-O_2 saturation. Thus, the Bohr and Haldane effects are linked functions, as has been recognized by the classic Wyman linkage equation:

$$(\log P_{O_2}/\text{pH})Y = (H^+/Y)\text{pH} \quad [3]$$

where Y refers to Hb-O_2 saturation and H^+ refers to the number of H^+ bound per heme molecule. Assuming that the shape of the OEC is symmetrical and H^+ release is linear as Hb binds O_2, which is often the case in vertebrates (but not always, as described below in section Nonlinear Bohr–Haldane Effect), the linkage equation is often reduced to the following:

$$-\Delta\log P_{50}/\Delta\text{pH} = \Delta H^+ \quad [4]$$

Thus, the Bohr and Haldane coefficients are numerically equivalent and will be referred to as the Bohr–Haldane coefficient and reported as a positive value from this point forward. It is important to note that this relationship has been experimentally validated as well. Air-breathing animals typically have moderate Bohr–Haldane coefficients (ie, 0.35), while most teleosts have relatively large Bohr–Haldane coefficients (0.5–>1.0). The numeric value has large implications for the nature of the interaction between O_2 and CO_2 exchange in vivo, as described in the following section.

Theoretical Bohr–Haldane Coefficient Optimal for Oxygen Delivery

The potential benefit to tissue O_2 delivery that is associated with the Bohr effect can be quantified as the product of the pH change during blood transit through a tissue (arterial–venous pH change (pH_{a-v})) and the magnitude of the Bohr–Haldane coefficient. A large Bohr coefficient is often assumed to convey a greater benefit to tissue O_2 delivery, but this will only be true if the pH change described above is sufficient. Associated with a large Bohr effect is a large Haldane coefficient; therefore, upon deoxygenation, Hb will bind H^+, thereby reducing the magnitude of the pH_{a-v} and consequently the expression of the Bohr effect at the tissues. In 1983, Lapennas conducted an analysis to determine the optimal Bohr coefficient for O_2 delivery under steady-state conditions taking into account the pH sensitivity of Hb associated with a given Bohr coefficient and the resulting pH_{a-v} that would occur in the face of the numerically identical Haldane coefficient. Lapennas concluded that the optimal Bohr coefficient for O_2 delivery under steady-state conditions (and with many assumptions) is $0.5 \times$ respiratory quotient (RQ; moles of CO_2 produced per mole of O_2 consumed). This represents a compromise between pH sensitivity of the Hb and the resulting pH change that occurs during capillary blood transit. Most animals have RQ values of between 0.7 and 1.0 and thus Lapennas' optimal Bohr coefficient is 0.35–0.5, which is very close to the value found in air-breathing vertebrates (0.35). Thus, he concluded that the Hbs of air-breathing vertebrates have been optimized for O_2 delivery (**Fig. 1**). Given that most teleost fish possess Bohr–Haldane coefficients much greater than $0.5 \times$ RQ, it has been assumed that, under steady-state conditions in most tissues, fish Hbs may be optimized for CO_2 transport and acid–base homeostasis rather than tissue O_2 delivery. This clearly does not apply to the unique structures within the swimbladder and eye, where there exists a tremendous potential for generating and localizing an acidosis, which in conjunction with the Root effect and associated large Bohr–Haldane coefficients generates incredibly high O_2 tensions. However, in other tissues, given Lapennas' assumptions, a very large Bohr–Haldane coefficient would not benefit tissue O_2 delivery. However, Lapennas' analyses do serve as an interesting framework for hypothesizing how different Bohr–Haldane coefficients within and between species may influence the interaction between O_2 and CO_2 exchange in vivo.

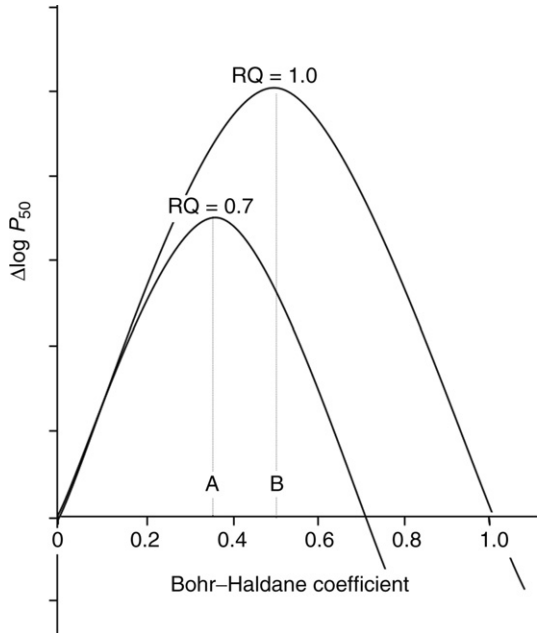

Fig. 1 The optimal Bohr–Haldane coefficient: theoretical Bohr shifts, as described by a change in P_{50} (Δlog P_{50}) during blood capillary transit using two respiratory quotients (RQs). Units have been omitted intentionally from the *y*-axis, because the magnitude of this response will vary by species, depending on Hb buffer values. A and B indicate Bohr–Haldane coefficients optimal for O_2 delivery for RQ values of 0.7 and 1.0, respectively. Each curve crosses the *x*-axis at both zero and the RQ, two points at which Lapennas suggests there will be no benefit to O_2 delivery associated with the Bohr–Haldane effect. Modified from Lapennas, G.N., 1983. The magnitude of the Bohr coefficient: Optimal for oxygen delivery. Respiration Physiology 54(2), 161–172.

Nonlinear Bohr–Haldane Effect Within the OEC

The assumption for many models depicting vertebrate Hb function is that the magnitude of the Bohr–Haldane coefficient is relatively constant across the entire OEC. Although this may be true for most air-breathing vertebrates, it is probably not the case in most fish species, where most of the Bohr–Haldane effect occurs in the upper reaches of the OEC (between 50 and 100% Hb-O_2 saturation) with very little expression below 50% Hb-O_2 saturation. The nonlinear Bohr–Haldane effect is typically associated with species that possess Root-effect Hbs, perhaps suggesting that nonlinearity is common among teleost species. When fish use different regions of the OEC for gas exchange in vivo, implications for a nonlinear Bohr–Haldane effect influencing the interaction between O_2 and CO_2 exchange become interesting.

The entire Bohr–Haldane effect may be exploited in resting fish, where venous Hb-O_2 saturation levels rarely fall below 50%. In resting rainbow trout (*Oncorhynchus mykiss*), the Bohr–Haldane coefficient calculated over the region of the OEC used in vivo is approximately 1.0, a value close to RQ. Therefore, CO_2 excretion at the gills and CO_2 and acid–base transport at the tissues will be maintained because Hb will bind all H^+ released from the tissues during O_2 delivery. However, when fish are forced to swim, the arterial–venous O_2 difference increases, requiring a greater region of the OEC to be used for gas exchange. The magnitude of the Bohr–Haldane coefficient calculated over the region of the OEC used for gas exchange during exercise is reduced to a value of 0.4–0.5, remarkably close to the value deemed optimal for O_2 delivery by Lapennas. Accordingly, during periods of increased activity and therefore muscle O_2 demand, the nonlinear Bohr–Haldane effect may be important for optimizing O_2 delivery. Thus, the nature of the interaction between O_2 and CO_2 exchange, if a nonlinear Bohr–Haldane effect is present, changes with exercise intensity and the region of the OEC exploited for gas exchange.

Implications of Non-Steady-State Conditions for the Interaction Between O_2 and CO_2 Exchange

Most of the discussion to this point has assumed steady-state, equilibrium conditions; however, it is unlikely that such conditions exist in vivo because gas exchange consists of a complex combination of blood flow, boundary layers, chemical reactions, and diffusion gradients. For example, if CO_2 from the tissues diffuses into the blood faster than O_2 diffuses to the tissues, a large Bohr–Haldane coefficient could facilitate O_2 delivery during blood capillary transit. The only way to determine whether this occurs in vivo is by direct measurement, which is very difficult. To date, only two studies have monitored real time tissue P_{O_2} in a teleost via fiber optic optodes implanted in the red muscle of resting rainbow trout. Results from both studies indicate a much higher

tissue P_{O_2} than in other vertebrates (eg, air-breathing mammals). Despite an in vivo Bohr–Haldane coefficient of 1.0 under resting conditions (far greater than the optimal value determined as described above), it could be that general O_2 delivery is enhanced in rainbow trout, which has been recently demonstrated, and perhaps other fish species. Clearly, additional studies of this nature are required to investigate this further.

In fish, the greatest disequilibrium state at the level of the RBC is likely associated with catecholamine-stimulated Na^+/H^+ exchange. During stress, or when Hb-O_2 saturation falls below 50%, metabolic CO_2 and H^+ production may be elevated resulting in a reduction in blood pH. Catecholamines such as adrenaline and noradrenaline are released into the circulation and bind to β-adrenergic receptors on the RBC membrane. Through adenylate cyclase, which activates 3′,5′-cyclic monophosphate (cAMP), the β-adrenergic Na^+/H^+ exchange (βNHE) on the RBC membrane is activated. During an acidosis, the carbonic anhydrase (CA)-catalyzed hydration of CO_2 inside the RBC produces H^+ and HCO_3^-. However, the activated βNHE pumps out H^+ in exchange for Na^+ and HCO_3^- is removed via anion exchange for Cl^-, albeit at a slower rate. The result is an increase in intracellular pH (pH_i) and therefore Hb-O_2 affinity, but at the cost of decreasing plasma pH. The osmotic gradient generated by Na^+ and Cl^- influx activates the Na^+, K^+ pump, and osmotically obliged water enters the RBC, causing the cell to swell. This process is thought to have evolved to protect O_2 uptake at the respiratory surface during a general acidosis in species possessing Root effect Hbs, where an acidosis drastically decreases not only Hb affinity but also carrying capacity for O_2. However, this is also an example of a disequilibrium state where O_2 and CO_2 transport dynamics do not follow the steady-state conditions assumed in previous models.

The adrenergically activated βNHE elevates pH_i but only in the absence of CA activity in the plasma. If CA were accessible to the plasma, H^+ would combine with HCO_3^- to form CO_2 at a catalyzed rate and CO_2 would back-diffuse into the RBC, therefore effectively short-circuiting βNHE activity and decreasing pH_i (**Fig. 2**). Fish lack plasma-accessible CA at the gills, and thus adrenergically activated βNHE can protect O_2 uptake at the respiratory surface; however, CA may be plasma-accessible in some tissues, where the CAIV isoform is bound to endothelial cells and plasma oriented. When βNHE is activated during stress, and blood passes through capillaries that possess plasma accessible CA, RBC βNHE activity is short-circuited, resulting in a much larger pH_{a-v} than would otherwise occur, greatly facilitating O_2 delivery to the tissues.

Short-circuiting of RBC βNHE function has been validated in rainbow trout in vitro. When CA was added to adrenergically stimulated, mildly acidified RBCs in a closed system, the result was a rapid acidification of RBC pH_i and a significant increase in plasma P_{O_2}. To verify this effect in vivo, rainbow trout were implanted with a fiber optic O_2 optode into the red muscle, and indeed, tissue P_{O_2} increased by 65% over resting levels when the fish were subjected to a mild acid–base disturbance induced by elevated environmental CO_2 (hypercarbia). This increase in P_{O_2}, was abolished after the injection of a membrane impermeable CA inhibitor into the blood, thus supporting the role of plasma accessible CA in increasing tissue P_{O_2}. Recent work on coho

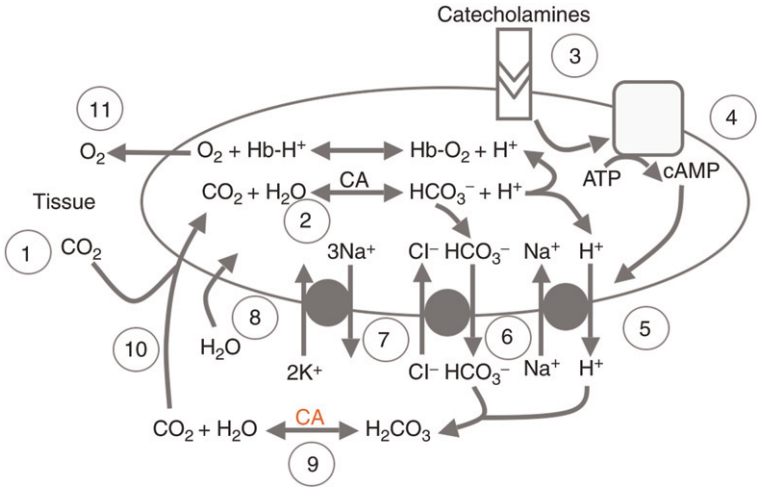

Fig. 2 Short-circuiting the β-adrenergic response at the red blood cell: With the advent of an acidosis or when metabolic CO_2 production is high, the red blood cell (RBC) intracellular pH (pH_i) can decrease substantially. Metabolic CO_2 enters the RBC (1) and is catalyzed by carbonic anhydrase (CA) to form HCO_3^- and H^+ (2), the latter of which will bind to hemoglobin (Hb), thus releasing O_2. Catecholamines such as adrenaline and noradrenaline are released into the circulation and bind to receptors on the RBC membrane (3), activating adenylate cyclase and 3′,5′-cyclic monophosphate (cAMP) (4), which activates the Na^+, H^+ exchanger (βNHE) (5). As protons (H^+) are removed from the RBC in exchange for Na^+, pH_i increases and Hb-O_2 affinity is restored. Bicarbonate (HCO_3^-) is removed from the RBC in exchange for Cl^- via the anion exchanger (6), resulting in an osmotic gradient that activates the Na^+, K^+ pump (7). Osmotically obliged water enters the cell (8) resulting in RBC swelling. As long as βNHE rates are high and CA is not accessible to the plasma, this results in an increase in RBC pH_i. However if CA is plasma accessible (9), which may occur in the tissues, H^+ and HCO_3^- removed from the RBC would form CO_2, at a catalyzed rate, and the CO_2 would back-diffuse into the RBC, thus re-acidifying it (10). This would short-circuit the original protective function of the βNHE mechanism and favor O_2 unloading from the Hb (11), which in the case of Root effect Hbs, could be substantial and could enhance O_2 delivery to tissues possessing plasma-accessible CA.

salmon (*Oncorhynchus kisutch*), a closely related teleost, verifies via immunohistochemistry, that CA may indeed be anchored to the endothelium in tissue capillaries, including those of red muscle, but is absent at the gill and the major blood vessels. This lends further support to an O_2 delivery model in teleosts, in which plasma accessible CA takes advantage of pH disequilibrium states across the RBC membrane to enhance O_2 unloading. Further studies have localized CAIV – a membrane-bound, plasma accessible isoform found in other vertebrates – in the atria of coho salmon hearts using a combined histochemical and genetic approach, and by measuring the rates of CO_2 dehydration in situ, it was determined that the enzyme is accessible to the plasma. Therefore, plasma-accessible CA may also be important in enhancing O_2 delivery to the avascular spongy myocardium of the heart, which would otherwise rely solely on the venous return that is notably O_2-poor.

Despite having a large Bohr–Haldane effect, salmonids (and probably other teleosts) may be able to enhance tissue O_2 delivery by exploiting pH disequilibrium states across the RBC membrane. This system could operate with every pass through the tissues, effectively harnessing a general blood acidosis to maximize the pH_{a-v} within the RBC. The resulting increase in O_2 unloading would be localized to tissues that possess or those downstream of plasma-accessible CA. The potential of such a system to increase O_2 unloading has been quantified using a modeling approach that, in rainbow trout, predicts a doubling of tissue-O_2 delivery during exercise and a tripling during conditions of severe hypoxia. In contrast, the O_2 delivery system in air-breathing vertebrates (including humans), which relies on a Bohr effect alone, may only enhance O_2 unloading from Hb by less than 2%.

Concluding Remarks

The intricate relationship between O_2 and CO_2 exchange has been described in past models that assume equilibrium conditions, which link the Bohr–Haldane relationship to the RQ in order to make predictions as to optimal O_2 delivery or CO_2 transport and acid–base homeostasis. For many teleost fishes, Lapennas' theory would predict that the large Bohr-Haldane coefficients are detrimental to tissue O_2 delivery. Yet, in vivo measurements confirm elevated muscle P_{O_2} in rainbow trout compared to air-breathing vertebrates, suggesting that a mechanism that relies on the activity of plasma accessible CA is in place to enhance tissue O_2 delivery. Equilibrium conditions probably never occur in vivo, and it appears that teleosts may harness pH disequilibria states across the RBC membrane to create the pH_{a-v} necessary to fully exploit the pH sensitivity of Root-effect Hbs. In addition, possessing a nonlinear Bohr–Haldane effect allows different parts of the OEC to be utilized under different conditions. Therefore, during non-steady-state conditions, such as intense exercise or hypoxia exposure, adrenergic stimulation of RBC βNHE creates an acid–base disequilibrium that is short-circuited by plasma-accessible CA in select locations within metabolizing tissue, thereby facilitating O_2 unloading to a much greater degree than what is attainable in air-breathing vertebrates. Root-effect Hbs evolved long before the eye and swimbladder *retia*, structures typically associated with this unique Hb pH-sensitivity, perhaps suggesting that Root effect Hbs were initially selected for enhancing general O_2 delivery and then later co-opted for these specialized tissues.

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