Gas transport and exchange: Interaction between O_2 and CO_2 exchange

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Glossary

Arterial Adjective pertaining to blood that has been oxygenated.
Bohr effect Effect of the proton 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 binding to hemoglobin (as a function of oxygenation).
Hypercarbia High levels of carbon dioxide in water or air.
P_{50} The oxygen partial pressure at half-maximal oxygen saturation of blood or hemoglobin.
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 Property of hemoglobins in some fishes such that, in the presence of acid, it is impossible for hemoglobin molecules to be completely saturated with oxygen, even at extremely high oxygen partial pressures.
Venous Adjective pertaining to blood that has passed through tissues having had some oxygen removed for metabolism.

Abstract

The interaction between O_2 and CO_2 in the general circulation of fish exists at the level of hemoglobin within the red blood cell, and is determined largely by the magnitude of the Bohr and Haldane effects. Assuming steady-state conditions, a Bohr–Haldane coefficient of 0.35–0.5 (0.5 × the respiratory quotient, RQ) is optimal for tissue O_2 delivery (excluding the swimbladder and eye), and greater values may be important for CO_2 excretion and acid–base homeostasis. Many teleosts possess a nonlinear Bohr–Haldane coefficient over the oxygen-equilibrium curve (OEC), which alters the nature of the interaction when different regions of the OEC are used for gas exchange. Recently, it has been discovered that Bohr–Haldane coefficients close to RQ (typically 0.7–1.0) may play an important role in facilitating tissue O_2 delivery *in vivo*, likely due to the elimination of large disequilibrium states in the blood due to the presence of membrane bound plasma accessible carbonic anhydrase.

Introduction

All animals produce about the same amount of CO_2 as O_2 consumed through the process of metabolism. O_2 is taken up from the environment and delivered to the tissues by the blood, and CO_2 is released by the tissues and transported by the blood for release to the environment. Hemoglobin (Hb), encapsulated within the red blood cell (RBC), plays a vital role in both O_2 and CO_2 transport in the blood of all vertebrates (with the exception of icefish, the only vertebrate 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) facilitates CO_2 removal through the Haldane effect, where Hb-oxygenation releases H^+'s that combine with HCO_3^- to form CO_2, which diffuses into the environment. 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 at the level of Hb within the RBC, which is 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 fish 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 fishes (teleosts) possess large Bohr and Haldane effects (as well as a Root effect, where oxygen-carrying capacity of the blood is reduced at low pH) and low Hb-buffer values, collectively resulting in tightly coupled O_2 and CO_2 exchange. Furthermore, many teleost fishes exhibit a nonlinear Bohr and Haldane effect over the region of the oxygen-equilibrium curve (OEC), which has 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 Hb for O_2 is affected for a given change in the H^+ concentration (pH) of the blood. It is calculated as follows:

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

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^+'s 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^+'s 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:

$$(\log Po_2/\text{pH})Y = (H^+/Y)\text{pH} \quad [3]$$

where Y refers to Hb-O_2 saturation and H^+ refers to the number of protons bound per heme molecule. Assuming that the shape of the OEC is symmetrical and H^+ release is linear with Hb-binding 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:

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

The point is that 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. Further, this relationship has been experimentally validated. Air-breathing animals typically have moderate Bohr–Haldane coefficients (i.e., 0.35), while most teleosts have relatively large Bohr–Haldane coefficients (0.5 to >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 associated with the Bohr effect is quantified as the product of the pH change associated with metabolic CO_2 production during blood transit through a tissue (arterial–venous pH change (pH_{a-v})) and the magnitude of the Bohr–Haldane coefficient. A large Bohr effect 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 effect; therefore, upon deoxygenation, Hb will bind H^+'s, 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. Assuming that the pH_{a-v} arises from tissue CO_2 production (and associated conversion to HCO_3^- and H^+) and that most animals have a tissue respiratory quotient (RQ) between 0.7 and 1.0 (moles of CO_2 produced per mole of O_2 consumed), a Bohr–Haldane coefficient of 0 will result in the largest pH change within the tissues. This would be due to the absent Haldane effect and associated H^+ binding upon deoxygenation, but would have no effect on O_2 delivery due to the lack of pH sensitivity of the Hb (i.e., no Bohr effect). Conversely, a Bohr–Haldane coefficient equivalent to the RQ (e.g., 1.0) will result in no pH change due

to the increased H^+ binding associated with the large Haldane effect. That is, all protons produced by CO_2 production at the tissues will be bound by Hb as O_2 is released to the tissues, and, despite the presence of a pH-sensitive Hb with a large Bohr effect, there may be no benefit to O_2 delivery due to the lack of pH_{a-v}. Furthermore, a Bohr–Haldane coefficient greater than RQ may result in a reverse pH_{a-v}, during blood transit, which actually could impair tissue-O_2 delivery.

Lapennas determined that the optimal Bohr coefficient for O_2 delivery under steady-state conditions (and with many assumptions) is $0.5 \times RQ$. This represents a compromise between pH sensitivity of the Hb and the resulting pH change that occurs during capillary blood transit. Because many air-breathing vertebrates have Bohr–Haldane coefficients of 0.35 (which is very close to optimal if RQ is assumed to be 0.7), he concluded that their Hbs 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-oxygen delivery. Despite Lapennas' many assumptions, his analyses 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*.

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 not the case in several 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 teleosts. 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

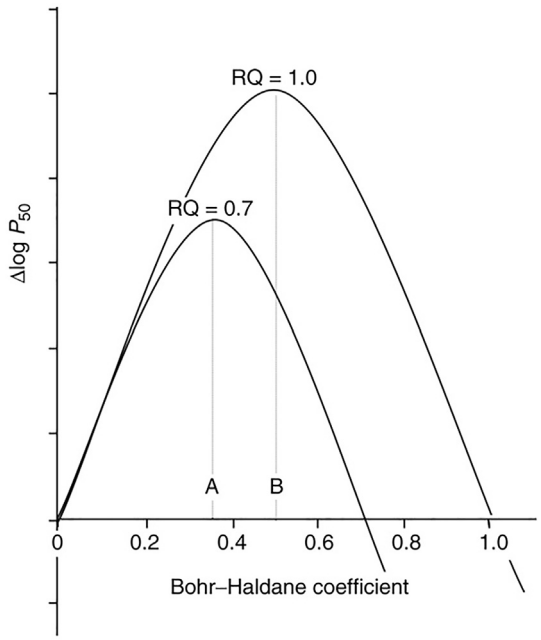

Fig. 1 The optimal Bohr–Haldane coefficient: theoretical Bohr shifts, as described by a change in P_{50} ($\Delta\log P_{50}$) during blood capillary transit using two respiratory quotients (RQs). Units have been omitted intentionally from the *y*-axis, because the magnitude of this response will vary 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 GN (1983) The magnitude of the Bohr coefficient: Optimal for oxygen delivery. *Respiration Physiology* 54(2): 161–172.

be maintained because Hb will bind all H^+'s 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. 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. In the only published study to date where this has been conducted in fish, P_{O_2} was measured in real time via an optode implanted in the red muscle of resting rainbow trout. Results indicate a much higher tissue P_{O_2} than in 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, 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. 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. The carbonic anhydrase (CA)-catalyzed hydration of CO_2 inside the RBC produces H^+ and HCO_3^-; the former are removed in exchange for Na^+ via the βNHE to restore pH_i and therefore Hb-O_2 affinity, but at the cost of decreasing plasma pH. The HCO_3^- is removed via anion exchange for Cl^-. The resulting osmotic gradient 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's affinity but also carrying capacity for O_2. However, this is also an example of a disequlibrium state where O_2 and CO_2 transport dynamics do not follow steady-state models.

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

Short-circuiting of RBC βNHE has been validated *in vitro*. When CA is added to adrenergically stimulated, mildly acidified RBCs in a closed system, it results in a rapid acidification of RBC pH_i and a dramatic increase in P_{O_2}. Accordingly, short-circuiting of RBC βNHE *in vivo* could potentially have large effects. In rainbow trout implanted with an O_2 optode in red muscle, which allows real-time tissue P_{O_2} monitoring, inducing a mild acid–base disturbance by exposing fish to elevated environmental CO_2 levels (hypercarbia) results in a significant increase in tissue P_{O_2}. However, when plasma-accessible CA is subsequently inhibited following the addition of a non-membrane-permeable CA inhibitor to the blood, the hypercarbia-induced increase in tissue P_{O_2} is abolished, indicating that short-circuiting of RBC βNHE may be operational *in vivo* and may greatly facilitate O_2 delivery. Therefore, although rainbow trout have a large Bohr–Haldane effect, a large pH_{a-v} in the RBC may occur via exploiting disequilibrium states *in vivo*. Furthermore, this system would operate with every pass through the tissues, effectively harnessing the general acidosis to create a RBC pH_{a-v}, which will be localized to tissues that possess plasma-accessible CA.

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. However, equilibrium conditions probably never occur *in vivo*. In addition, teleosts possess a large Bohr–Haldane coefficient, which according to Lapennas' theory may be detrimental to tissue O_2 delivery. Yet, real-time measurements confirm elevated muscle P_{O_2} in rainbow trout, suggesting that a mechanism is in place to enhance O_2 delivery in fish. Teleosts' unique Root-effect Hbs convey a great potential for O_2 delivery, and possessing a nonlinear Bohr–Haldane effect allows different parts of the OEC to be utilized under different conditions. Indeed, the potential exists, and during non-steady-state conditions such

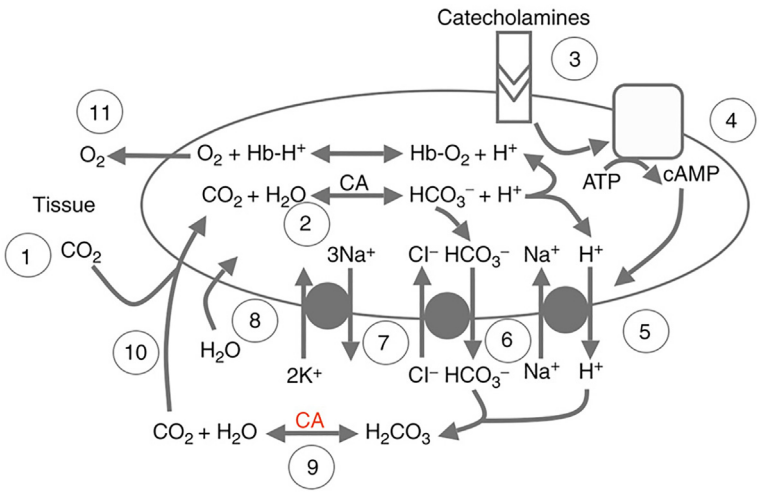

Fig. 2 Short-circuiting the β-adrenergic response at the red blood cell: With the advent of an acidosis or when metabolic CO_2 production is high, the red blood cell (RBC) intracellular pH (pH_i) can decrease substantially. Metabolic CO_2 enters the RBC (1) and is catalyzed by carbonic anhydrase (CA) to form HCO_3^- and H^+ (2), the latter of which will bind hemoglobin (Hb), thus releasing O_2. Catecholamines such as adrenaline and noradrenaline are released into the circulation to bind to receptors on the RBC membrane (3), activating adenlylate 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 pHi. However if CA is plasma accessible (9), which may occur in the tissues, H^+ and HCO_3^- removed from the RBC would be catalyzed to form CO_2 that would back-diffuse into the RBC (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 would be substantial, creating enhanced O_2 delivery localized to tissues possessing plasma-accessible CA.

as intense exercise or hypoxia exposure, adrenergic stimulation of the RBC βNHE creates the acid—base disequilibrium needed to maximize this potential. Plasma-accessible CA in select locations within metabolizing tissue may be localizing the acidosis by short-circuiting the βNHE, therefore facilitating O_2 delivery to tissues to a much greater degree than that in air-breathing vertebrates. Root-effect Hbs evolved long before the eye and swimbladder retia, structures typically associated with the unique pH-sensitive Hb, suggesting a general use for such great O_2 delivery potential. Along with the increase in and nonlinearity of the Bohr—Haldane effect, teleosts appear to greatly facilitate O_2 transport with only moderate whole-blood pH changes.

See Also: Acid-base regulation and hypercapnia: An introduction; Carbon dioxide transport and excretion; Cellular respiration; Evolution of the Bohr effect; Generation of the respiratory rhythm in fish; Metabolic rate depression as a mechanism for surviving hypoxia; Oxygen in the marine environment.

Further reading

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