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IN VIVO COMPARISON OF NON-GASEOUS METABOLITE AND OXYGEN TRANSPORT IN THE HEART

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INTRODUCTION

Oxygen transport has traditionally been approached as a specialized subject with little connection to the large amount of data on transport of other substances, equally essential for steady-state metabolism. Heuristically, there is no reason to expect a major difference but measurements of tissue PO_2 with oxygen electrodes in organs with high oxygen consumptions have yielded data which are incompatible with the classical Krogh-cylinder model of capillary-tissue oxygen transport. A number of alternative models, including diffusional shunting and flow heterogeneity, have been developed on the assumption that oxygen transport is a special case, with little or no consideration of the overall, nature of organ transport as reflected in the transport of other substances equally essential for metabolism. As we shall show, when examined in this light, oxygen transport is not essentially different from that of other substances. With the understanding afforded by this approach and recent developments based on it, future investigational effort can now be profitably directed at more complex problems, such as the role of impaired oxygen transport in certain pathological states of vital organs.

FREE FATTY ACIDS

In the case of the heart there is, we think, a direct analogy between the transport of free fatty acids (FFA) and oxygen. After fasting and during aerobic exercise, FFA are the major substrate for energy production in the heart. Like oxygen, FFA are highly extracted (up to 60% when circulating lactate is low) and like oxygen they are bound to a carrier in the blood (albumin, in the case of FFA, and hemoglobin, in the case of oxygen) Also, like oxygen it had been assumed that, being lipid soluble, FFA would diffuse so rapidly into the myocyte that venous outflow concentration would reflect the tissue concentration. Utilizing the multiple indicator dilution technique with labeled palmitate as an example of an FFA, Rose and Goresky (1976) refuted these assumptions. Labeled albumin was used as an intravascular reference and labeled sucrose as an inert marker which permeates the capillaries via the aqueous pores and distributes in the interstitial space. All three tracers were injected simultaneously in the coronary artery of an intact, working heart and venous samples were collected rapidly from the coronary sinus. The activity of each tracer was

measured in each sample and divided by the respective activity in the injection mixture, to give the normalized outflow concentration. Figure 1 shows two sets of data before and after intravenous lactate infusion. The form of the albumin outflow is determined solely by the distribution of large and small vessel transit times, of the sucrose, additionally, by the volume of the interstitial space, and of the palmitate, also by permeation into and metabolism within the myocyte. When intracellular sequestration is inhibited by providing an alternative substrate (lactate), more of the labeled FFA that entered the myocyte returns to the capillary. Thus, outflow of the labeled FFA is composed of two components, an early exiting portion which has never left the capillary and a later or returning component which is material which has left and which returns later in time.

On inspection of this data a few features are immediately apparent. First, the rate constant for capillary permeation of the FFA (which is roughly indicated by the ratio of albumin to palmitate at the peak of the curves) is only slightly greater than that for sucrose, a substance which permeates the capillary endothelium only via the aqueous pores. Second, most of the FFA remaining in the coronary sinus has not even left the capillary in its passage. Thirdly, there is no change in the throughput component when the net transcapillary flux of FFA was reduced by lactate competition.

Modeling of the processes involved in the exchange process revealed that, in the steady state, calculated intracellular concentrations of FFA were only one-twentieth of the plasma concentration. This prediction was verified a few years later by van der Vusse et al (1982) who measured tissue FFA concentrations in rapidly frozen samples of myocardium. The explanation is that, even for a substrate as important as FFA, the tissue concentration will be lower than the venous concentration when there is sequestration behind a relative barrier to diffusion (Goresky et al, 1983).

The nature of this barrier is revealed when tracer amounts of palmitate are dissolved in water and dextran is used as a reference, in order to eliminate albumin from the perfusion system in an isolated heart preparation (Little and Bassingthwaite, 1987). Figure 2 shows that when albumin is removed the extraction of FFA increases to 95% and the estimated rate constant for FFA diffusion from the capillary increases by an order of magnitude. It appears that, within physiologically attainable plasma FFA concentrations, the capacity to sequester FFA is limited only by the diffusion barrier. If we can assume that the permeability of the capillary endothelium was no different in the two situations, then the increase in the transport rate constant was due only to the elimination of albumin binding sites in the plasma within the capillary. The true permeability may be even higher than estimated from this data because it is impossible to dissolve even tracer amounts of FFA in water without formation of micelles, which would tend to limit diffusion. The appendix shows mathematically why an increase in the apparent vascular volume of distribution of a permeating substance will reduce the rate constant for permeation. This is a general phenomenon and applies to any substance with a large relative volume of distribution or binding capacity in the capillary. Of course, the best example of such a substance is oxygen.

OXYGEN

August Krogh (1919) imagined a capillary surrounded by a large cylindrical tissue space homogeneously consuming oxygen. While the model was useful in proving that physical diffusion of oxygen was sufficient to explain the delivery of oxygen to tissue at physiologic rates of oxygen consumption in resting skeletal muscle, the model did not include blood flow rate, capillary length or potential inhomogeneities in oxygen diffusion associated with membranes. Motivation for more complex modeling came from the accumulation of tissue PO_2 histograms from various organs under various conditions. As a general rule, the smaller

the polarographic electrode the more likely the PO_2 histogram will represent the true intracellular PO_2 . Whalen and co-workers (Whalen, 1973; Schubert et al, 1978) have been the only investigators to consistently use electrodes with tip diameters on the order of $1\mu m$. Electrodes larger than this are likely to interfere with the microcirculation or give a mixture of capillary and tissue rather than a true extravascular value. Neo-Krogh models with varying capillary geometries and diffusional interaction have been developed (Metzger, 1969; Grunewald and Sowa, 1978; Schubert et al., 1978) and predicted random PO_2 histograms have been compared with the data. There is agreement in cases of low oxygen consumption but not in the more interesting situations.

In the liver, an organ with a relatively large vascular space and discontinuous capillaries, tissue PO_2 measured with surface electrodes is between arterial and venous values (Matsumura et al, 1986). The earlier measurements of Kessler (1967) using penetrating $50\mu m$ electrodes probably were too low because the electrode interfered with local flow in the sponge-like liver. In the case of the heart, there is a major discrepancy between the predictions of neo-Krogh modeling and tissue PO_2 histograms. The problem is was succinctly described by Lubbers in 1982:

“We are able to show that...an oxygen pressure field can be characterized...by a PO_2 frequency histogram obtained from 100–200 local PO_2 measurements using classes of 5 mmHg...For example, in a dog with a PO_2 in the sinus coronarius of 18 mmHg, the maximum of the histogram is in the class of 10–20 mmHg (32%); 11% of values are below 10 mmHg, i.e. about 43% of PO_2 values are equal to or below the venous PO_2 . Since, due to the small capillary distances in the myocardium, [predicted] PO_2 [difference] between capillary and tissue is only in the range of 2–3 mmHg, this cannot be explained by a simple capillary model.”

Data obtained using smaller electrodes are even more anomalous (Whalen, 1973). The discrepancy between data and model is not resolved by inserting heterogeneity of capillary flows or capillary diffusional interaction into the standard model (Grunewald and Sowa, 1978).

Another set of observations also emphasizes the large gradient between venous and tissue PO_2 . In situ, the myocardium begins to fail mechanically and produce lactate when blood flow is reduced such that the PO_2 in the coronary sinus is 9–11 torr (Case, 1966). Isolated cardiac myocytes, however, maintain oxygen consumption down to a PO_2 of 0.2 torr (Wittenberg and Robinson, 1981). There are two orders of magnitude between the two definitions of hypoxia!

There are two and only two explanations for these anomalies; both necessitate a rejection of the classical Krogh model. Either there is a diffusional shunt at the level of the arterioles and venules which would lower arteriolar PO_2 and raise venous PO_2 relative to tissue PO_2 as proposed by Schubert et al (1978), or the diffusion rate of oxygen from blood to tissue is much less than that measured across a slab of muscle tissue.

There is a relatively easy way to discover which explanation is the correct one. If there were a large diffusional shunt, coronary sinus blood samples collected sequentially after a bolus of blood containing tracers for erythrocytes and oxygen was injected into a coronary artery would show tracer oxygen appearing before the erythrocytes, which would necessarily have to take the longer pathway through the tissue. On the other hand, if there were a resistance to diffusion the outflow pattern would resemble that of other substances such as FFA undergoing barrier-limited transport (Rose and Goresky, 1976). Using $^{18}O_2$, a heavy isotope of oxygen as the oxygen tracer we performed such an experiment (Rose and Goresky, 1985). The results are shown in Figure 3 and were unequivocal; there was no precession of oxygen

before the erythrocytes but there was evidence for a barrier between erythrocytes and mitochondria, probably at the level of the endothelial membrane. Previously, the permeability of continuous capillaries to oxygen had been measured in only one organ, the rete mirabile of the eel, which consists only of capillaries. During counter-current perfusion of this organ with erythrocyte-free, oxygenated buffer the endothelial barrier reduced the apparent diffusion coefficient of oxygen to 0.5% of its free diffusion coefficient in water (Rasio and Goresky, 1983); the permeability was about twice that for tracer water in the same system. In the heart, on the other hand, the relatively high but finite permeability of the endothelial cell membrane to oxygen is decreased by two orders of magnitude by the hemoglobin binding of tracer oxygen to yield an apparent rate constant for oxygen transfer no greater than that for the sodium ion, which diffuses only via aqueous pores in the capillary. The analogous experiment to the albumin-free FFA experiment would be a tracer oxygen experiment with hemoglobin free perfusate. Unfortunately, our quadrupole mass spectrometer is not sensitive enough to detect such small amounts of heavy oxygen as would dissolve in water against a background of the much more abundant, dissolved carbon dioxide. We would predict that when such data becomes available it will look something like that in Figure 2 if tracer oxygen is substituted for tracer FFA. The factor by which hemoglobin binding reduces the rate constant for oxygen transport can be calculated from knowledge of the ratio of the oxygen content of plasma to that of red cells in fully oxygenated blood, $0.3:20.0 = 0.015$. Now, since the ratio of sodium to oxygen rate constants is about 1.4 (Rose and Goresky, 1985), the ratio of oxygen to sodium capillary permeabilities is about 94. Thus, the large intravascular hemoglobin binding capacity reduces the rate constant for oxygen exchange by two orders of magnitude and the diffusional limitation offered by the capillary endothelial membrane is effectively magnified by this factor. This phenomenon was not envisioned by Krogh and has not been included in any of the previous attempts to model in vivo oxygen transport. However, it could have been predicted from the FFA data which has been available for more than ten years.

Modeling of the transport process also gives an estimate of the ratio of extravascular to intravascular oxygen concentrations. We estimate that the extravascular concentration is at least a factor of three lower than the blood concentration at any point along the capillary. This estimate is dependent on the details of the modeling which omits some aspects of oxygen transport such as nonlinear hemoglobin-oxygen binding and potentially zero-order sequestration. Data from hemoglobin-free, isolated hearts, in which myoglobin saturation is used to estimate intracellular PO_2 , suggest that the transcapillary gradient may be as much as two orders of magnitude (Araki et al, 1983). This preparation is, however, characterized by huge perfusion rates and interstitial edema and certainly does not accurately reflect the physiological situation. The data indicate that even if the transcapillary gradient is only one order of magnitude, the normal heart functions at an intracellular PO_2 no more than an order of magnitude above the critical PO_2 for isolated myocytes, which is estimated at about 0.2 mmHg (Wittenberg and Robinson, 1981). While the oxygen transport system is adequate for normal heart function, it is not unlikely that its limitations become critical when oxygen consumption is chronically increased, for example in valvular heart disease. In this situation the eventual deterioration in heart function which results in the symptoms of heart failure could be caused by some dysfunction related to chronic tissue hypoxia.

The large capillary-tissue oxygen concentration gradient predicted from our data in the heart was supported by data from exercising skeletal muscle obtained by Gayeski and Honig (1986) who measured oxygen saturation of myoglobin microcryophotometrically in cross sections of muscle fibers, freeze-clamped during twitch contraction. They found very low PO_2 's of about 2–3 mm Hg, much lower than the venous PO_2 , and exhibiting little intracellular gradient. If we can assume by analogy with the heart that there is no large vessel diffusional shunt then this data can be explained by a relative diffusion barrier at the

level of the endothelium. An alternative explanation has been proposed by Groebe and Thews (1986). They have proposed that the steep capillary-tissue gradient is the result of high flux densities through a “resistance layer” composed of endothelium and interstitial space which do not contain hemoglobin or myoglobin. If this formulation is correct then any apparent barrier should disappear at low rates of oxygen consumption and outflow dilution curves should conform to the flow-limited or delayed-wave model of exchange (Goresky et al, 1970). We believe that this is unlikely because the data from the heart suggest that when net transcapillary flux is lowered in the case of both FFA and oxygen there is no change in the apparent throughput component; the barrier appears to present regardless of the rate of transport. A definitive answer must await outflow dilution data from resting skeletal muscle or unloaded heart.

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APPENDIX

Mathematical description of the effect of intravascular binding on uptake rate constant and transit time

The conservation equation for a single capillary and an associated extravascular space in which oxygen is bound reversibly to moving red cells is

$$\frac{\partial u}{\partial t} + W \frac{\partial u}{\partial x} + \gamma \frac{\partial v}{\partial t} + R_c \beta \left(\frac{\partial y}{\partial t} + W \frac{\partial y}{\partial x} \right) + R_m \theta \frac{\partial z}{\partial t} + R_m \theta k_3 z = 0,$$

where

u is the plasma concentration in the plugs of plasma between the red cells,

v is the plasma concentration in the small annulus of plasma around the red cells,

y is the concentration in the red cells,

z is the concentration in the extravascular space,

W is the velocity of the red cells,

β is the ratio of red cell volume to moving plasma volume such that hematocrit = $\beta / (1 + \beta)$,

γ describes the ratio of the volume of plasma in excess of that moving plug-like with the red cells,

θ is the ratio of extravascular volume to plasma volume for oxygen,

R_c is the ratio of red cell to plasma oxygen concentration,

R_m is the ratio of extravascular to plasma oxygen concentration in an equilibrium situation, and,

k_3 is the first-order rate constant for irreversible sequestration within the extravascular space.

Note that oxygen uptake is close to zero-order in the physiological situation and that the sigmoidal nature of the hemoglobin-oxygen dissociation curve has not been accounted for. Since this simplified model appears to explain the available data to a first approximation, these refinements have not been included. As more precise data becomes available, their inclusion may become necessary.

The mass balance equation for the extravascular space is

$$\frac{\partial z}{\partial t} - k_1 u + k_2 z + k_3 z = 0,$$

where

k_1 is the rate constant for outward transport from the capillary and,

k_2 is the rate constant for inward transport to the capillary from the extravascular space.

The details of the simultaneous solution of these two partial differential equations will not be given here because of space limitations. The final result is,

$$u(x, t) = \frac{q_0}{F_c} \cdot e^{-\frac{k_1 R_m \theta}{(1+R_c \beta)} \frac{x}{W}} \cdot \delta \left(t - \frac{(1+\gamma+R_c \beta)}{1+R_c \beta} \frac{x}{W} \right) + \frac{q_0}{F_c} \cdot e^{-\frac{k_1 R_m \theta}{(1+R_c \beta)} \frac{x}{W}} \cdot e^{-(k_2+k_3) \left(t - \frac{(1+\gamma+R_c \beta)}{(1+R_c \beta)} \frac{x}{W} \right)} \cdot \sum_{n=1}^{\infty} \frac{\left(\frac{k_1 k_2 R_m \theta}{(1+R_c \beta)} \frac{x}{W} \right)^n \left(t - \frac{(1+\gamma+R_c \beta)}{(1+R_c \beta)} \frac{x}{W} \right)^{n-1}}{n!(n-1)!} \cdot S \left(t - \frac{(1+\gamma+R_c \beta)}{(1+R_c \beta)} \frac{x}{W} \right),$$

where q_0 is the amount of tracer injected and F_c is the flow rate in the capillary.

The first term in the above expression represents that part of the inflow that does not leave the capillary and the second term is the part which has left and returns later in time. The important point here is that the rate constant for capillary exchange, k_1 , is modified by a factor, $R_m \theta / (1 + R_c \beta)$, which, for oxygen, is roughly the ratio of plasma to red cell oxygen contents.

In the case of the FFA all of the tracer is confined to the plasma phase so that $\gamma = 0$. Also, the effect of binding to albumin can be modeled by substituting

$$\frac{[\text{free FFA}]}{[\text{total FFA}]} = \frac{1}{1+R_c \beta}.$$

The FFA also bind to albumin in the interstitial space and consequently encounter a resistance at the sarcolemmal membrane which is not included in this simplified model.

Figure 1.

Normalized coronary sinus outflow dilution curves after simultaneous injection of labeled albumin, sucrose and palmitate into the coronary artery of an anesthetized dog before and after the infusion of lactate. The lower panels are the corresponding log ratio curves where C_{Ref} represents albumin and C_{Diff} represents sucrose or palmitate. Reproduced from Rose, C.P., Goresky, C.A., 1977, Constraints on the uptake of labeled palmitate by the heart: the barriers at the capillary and sarcolemmal surfaces and the control of intracellular sequestration, *Circ. Res.*, 41: 534–545 by permission of the American Heart Association, Inc.

Figure 2.

Normalized coronary sinus outflow dilution curves after simultaneous injection of labeled dextran and palmitate into the aortic root of an isolated rabbit heart preparation. Compare with Figure 1. The absence of albumin in the perfusion fluid caused a large increase in the extraction of palmitate. Unpublished data of Bassingthwaighe and Little.

Figure 3.

Normalized coronary sinus outflow curves after simultaneous injection of labeled red cells, albumin, sodium ion and oxygen into the coronary artery of an anesthetized dog. Note that the peak extraction of oxygen is no greater than that of sodium ion and that reduction of oxygen consumption after β -blockade causes a change only in the returning component. Reproduced from Rose, CP., Goresky, C.A., 1985, Limitations of tracer oxygen uptake in the canine coronary circulation, *Circ. Res.*, 56: 57–71 by permission of the American Heart Association, Inc.