CROSSTALK

CrossTalk proposal: Physiological CO_2 exchange can depend on membrane channels

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Introduction

Since the discovery that CO_2 passes through aquaporin-1 (AQP1; Nakhoul *et al.* 1998; Cooper & Boron, 1998), the importance of channel- *vs.* lipid-mediated gas transport has often been portrayed as an either/or issue. However, depending upon physiological context, the role of channels may be insignificant or dominant.

In a landmark study, Mitchell (1830) examined gas permeation across barriers of natural rubber or animal tissue, rank-ordered the 'relative facility of transmission' of several gases, and recognized that these move independently of one another in a mechanism dependent upon 'infiltration' (i.e. solubility) in the organic molecular barrier – the first statement of 'solubility theory'. Later, Graham showed that permeation across rubber membranes depends on not only solubility but also diffusion through the barrier (Graham, 1866) – the first statement of 'solubility–diffusion theory'. Meanwhile, Fick proposed his law of mass diffusion, which Wroblewski combined with Henry's law to produce our modern transport equation (Wroblewski, 1879):

$$J = \frac{Ds}{l}\Delta p$$

Here J is flux, D the diffusion constant in the barrier, s solubility in the barrier, l barrier thickness and Δp trans-barrier partial pressure difference.

In the late 1890s, Overton used solubility theory to develop his revolutionary hypothesis that the boundary layer of the cytoplasm ('Grenzschicht des Protoplasten') – now termed the plasma membrane – is impregnated with lipids. However, modern reference to 'Overton's rule'

$$\text{True membrane permeability to substance } X (\text{i.e., } P_{M,X}) \propto s$$

is problematic because (1) the 'solubility' rule is really Mitchell's and ignores both (2) D and (3) membrane proteins, which themselves impact J in three ways. First, in the plane of the membrane, proteins impermeable to X displace and organize nearby lipids, reducing $P_{M,X}$ (Wang *et al.* 2007; Boron, 2010). Second, transporters and channels carry a wide range of lipid-soluble solutes (Al-Awqati, 1999). Thus, lipid solubility does not prove permeation via membrane lipid. Third, exomembranous portions of integral membrane proteins can almost completely cover some membranes (Takamori *et al.* 2006). Boron proposed the 'access–solubility–diffusion–egress theory' to account for the resistance of these proteins, and the ordering of water near charged lipid head-groups, to the entry of a substance into (or exit from) membrane lipid.

The first clear experimental data opposing the solubility–diffusion theory was the demonstration that gastric gland apical membranes have no measurable P_{M,CO_2} (Waisbren *et al.* 1994). In artificial lipid bilayers, a major determinant of P_{M,CO_2} may be membrane lipid cholesterol content ($C_{M,chol}$; Itel *et al.* 2012; Kai & Kaldenhoff, 2014). In one study, raising $C_{M,chol}$ from 0 to 20% lowered P_{M,CO_2} by ≥10-fold; raising $C_{M,chol}$ from 20 to 70% decreased P_{M,CO_2} by an additional 10-fold (Itel *et al.* 2012), predominantly due to a decrease in D rather than s. The CO_2-impermeable plant aquaporin NtPIP2;1 reduces P_{M,CO_2} of artificial membranes (Kai & Kaldenhoff, 2014) by displacing lipids, further reducing 'background' CO_2 permeability. True membrane permeability depends on two parallel pathways that sum like parallel electrical conductances: $P_{M,CO_2} = P_{Back,CO_2} + P_{Channel,CO_2}$. In series with the membrane are unconvected layers (ULs; Fig. 1*A*) that reduce the measured apparent membrane permeability (P_{M',CO_2}):

$$\frac{1}{P_{M',CO_2}} = \frac{1}{P_{UL,CO_2}} + \frac{1}{P_{Back,CO_2} + P_{Channel,CO_2}}$$

Thus, channels likely contribute more when ULs are thin and P_{Back,CO_2} is low (as in red blood cells (RBCs)), but less when ULs are thick and/or P_{Back,CO_2} is high (as in some solid tumour models).

Options in cell membrane design

P_{M,CO_2} can span many orders of magnitude. At one end of the spectrum are artificial lipid bilayers, typically loosely packed lipids containing as much as 30% of the solvent *n*-decane (Fig. 1*A*). Here, $P_{M,CO_2} = P_{Back,CO_2}$, high enough to make measurement difficult.

Further along the spectrum are plasma membranes from cells with modest $C_{M,chol}$

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and abundant integral membrane proteins (Fig. 1*B*). Examples include MFC7 breast tumour cells and ascites tumour cells, with $C_{M,chol}$ of ∼25% (Haeffner *et al.* 1984; Todor *et al.* 2012). With such a low $C_{M,chol}$, P_{Back,CO_2} could be sufficiently high that, even without channels, ascites tumour cells could accommodate their modest $\dot{V}_{CO_2}$ of 0.12 ml g^{-1} min^{-1} (Warburg, 1956), according to Endeward's analysis (2014).

Even further along the spectrum are Madin-Darby canine kidney (MDCK) cells, with an intermediate $C_{M,chol}$ (37%), no known gas channels, and a low P_{M,CO_2} (Itel *et al.* 2012) that presumably represents P_{Back,CO_2} and is sufficient to meet a low metabolic demand (Fig. 1*C*). Interestingly, depleting MDCK cells of cholesterol (i.e. raising P_{Back,CO_2}) or expressing AQP1 (i.e. raising $P_{Channel,CO_2}$) raises P_{M,CO_2}.

Falling into the same cholesterol content category (∼40%) as MDCK cell membranes are RBC membranes (Fig. 1*D*). Although RBCs have a low P_{Back,CO_2}, a high gas-channel content gives them a high P_{M,CO_2} (see below).

At the far end of the spectrum are proximal colon apical membranes, with $C_{M,chol}$ of 77%, consistent with the observed low CO_2 permeability (Fig. 1*E*; Endeward *et al.* 2014). Apical membranes of gastric glands have no measurable P_{M,CO_2} (Waisbren *et al.* 1994).

Gas channels

In 1998 Boron's laboratory identified the first family of gas channels by showing that

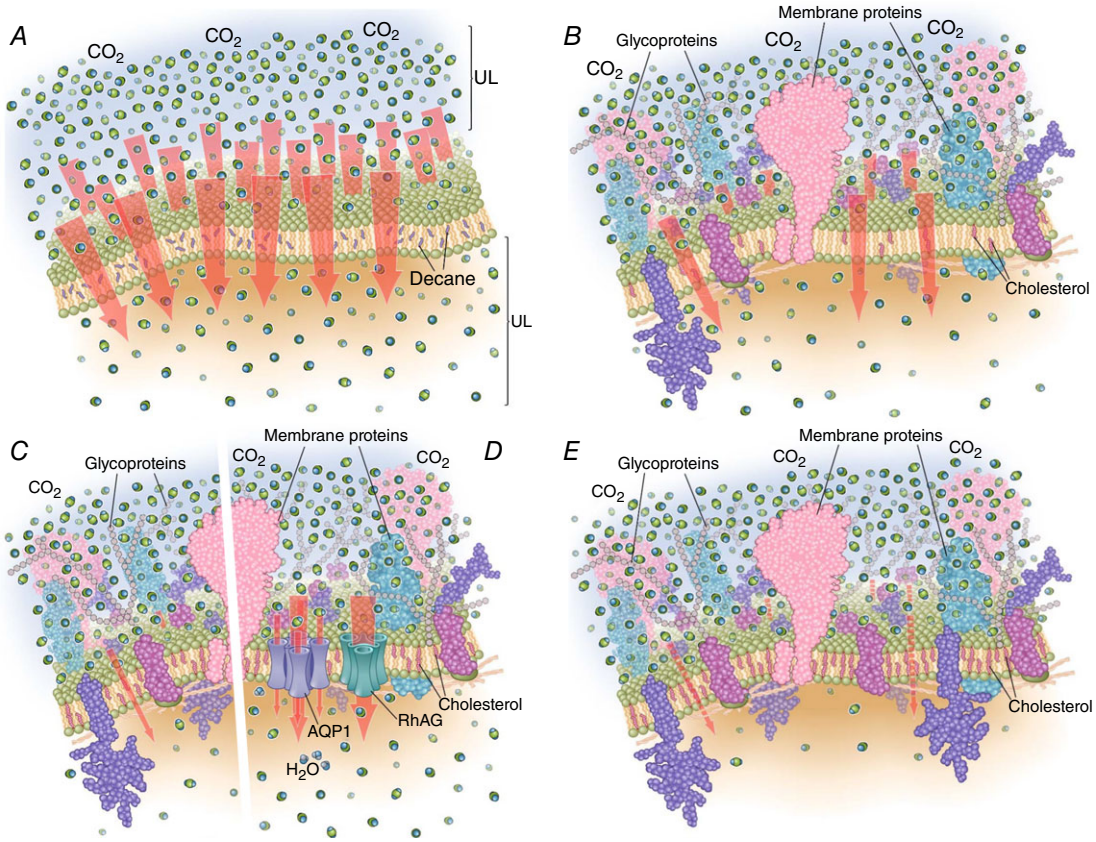

Figure 1. Models of CO_2 movement across membranes of artificial lipid bilayers (*A*), as well as plasma membranes with low cholesterol content (*B*), moderate cholesterol content without (*C*) and with (*D*) gas channels, and high cholesterol content without gas channels (*E*)
Membrane composition has dramatic effects on CO_2 permeability. Red arrows represent CO_2 flux. The density of CO_2 molecules on upper side of membrane (exaggerated by ∼5-fold, assuming a membrane thickness of 6 nm and 5% CO_2 at room temperature, for illustrative purposes) is the same in all examples; the densities above and below the membranes represent snapshots during the early moments following CO_2 addition to extracellular solution. The density of membrane proteins is less than indicated for synaptic vesicles by Takamori *et al.* (2006). In *A*, the artificial lipid bilayer contaminated with decane has an extremely high permeability to CO_2. The square brackets UL indicate the unconvected layers either side of the membrane. *B–E* show biological membranes with integral proteins and increasing levels of cholesterol, both of which reduce 'background' CO_2 permeability (i.e. not due to channels). In *B*, the presence of gas-impermeable proteins and low cholesterol concentrations (∼25% of membrane lipid) lowers CO_2 permeability and flux. In *C*, further increasing membrane cholesterol content (∼40%) dramatically decreases CO_2 permeability. In *D*, membranes (e.g. from RBCs) with the same content of integral membrane proteins and cholesterol as in *C* have a much higher CO_2 permeability because specialized channels such as AQP1 and the Rh-associated glycoprotein (RhAG) augment the passage of CO_2. In *E*, membranes (e.g. apical membranes of proximal colon) with extremely high cholesterol content (∼77%) are expected to have a very low CO_2 permeability.

CO_2 moves through AQP1, heterologously expressed in *Xenopus* oocytes (Nakhoul *et al.* 1998; Cooper & Boron, 1998). Both *p*-chloromercuriphenylsulfonic acid (pCMBS) (Cooper & Boron, 1998) and DIDS (Endeward *et al.* 2006) significantly reduce AQP1-dependent P_{M,CO_2}; because pCMBS but not DIDS reduces water permeability, these agents act via different pathways. AQP1 also conducts NH_3 (Nakhoul *et al.* 2001) and NO (Herrera *et al.* 2006).

Ripoche *et al.* (2004) and Khademi *et al.* (2004) identified another gas-channel family, the Rhesus (Rh) proteins, by demonstrating permeability to NH_3. Work with human RBCs showed that the Rh complex also conducts CO_2 (Endeward *et al.* 2008). Further studies show that each AQP and Rh protein exhibits a characteristic selectivity for CO_2 *vs.* NH_3, with some AQPs being impermeable to CO_2, NH_3, or both (Musa-Aziz *et al.* 2009; Geyer *et al.* 2013*b*,*c*).

Recently, Boron's group identified a third gas-channel family: the urea transporter UT-B, which is permeable to NH_3 (Geyer *et al.* 2013*a*). The known gas-channel families consist of physiologically active monomers surrounding a central structure that, for AQPs and Rhs, is a lipophilic pore. We hypothesize that some of these central pores conduct CO_2 or other dissolved gases, and that other families of gas channels remain to be identified – known proteins with previously unappreciated gas-selective pathways. Thus, arguing that particular membranes lack particular channels ignores the possibility of yet-to-be-discovered channels.

Physiological role

The first demonstrated physiological role for channels in gas transport was CO_2 uptake (driven by an exceeding small gradient) via NtAQP1 in tobacco plants during photosynthesis (Uehlein *et al.* 2003). Studies on human RBCs show that DIDS plus the genetic deletion of either AQP1 (Colton-null) or Rh complex reduces P_{M,CO_2} by ≥90% (Endeward *et al.* 2006, 2008), leaving, at most, 10% for CO_2 pathways through lipid. Thus, channels almost certainly make a physiologically important contribution to CO_2 exchange in pulmonary and systemic capillaries, particularly during conditions of short transit time, such as exercise (Endeward *et al.* 2014).

Channels could make significant contributions in other systems with high CO_2 fluxes and high AQP levels. Examples include alveolar type I pneumocytes (AQP5; Verkman *et al.* 2000), astrocytic endfeet/blood–brain barrier (AQP4; Nagelhus *et al.* 2004) and renal proximal tubules (AQP1; Schnermann *et al.* 1998).

In conclusion, channels contribute to P_{M,CO_2} on a sliding scale that depends on the balance of $P_{Channel,CO_2}$ *vs.* P_{Back,CO_2} and ULs. Moving the field forward, and knowing where cells sit on the sliding scale, will require understanding this balance by examining, in simple systems, how physiological ULs and altered membrane composition (i.e. the nature and number of lipids, non-channel proteins, channels) affect gas fluxes.

Call for comments

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Additional information

Competing interests

None declared.

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