Original Paper

CO₂ Permeability of Rat Hepatocytes and Relation of CO₂ Permeability to CO₂ Production

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Key Words

Rat hepatocytes • CO₂ permeability • Oxygen consumption • Intracellular carbonic anhydrase activity • Extracellular carbonic anhydrase inhibitor • Organic cation transporter 1 • Cholesterol • Gas channels

Abstract

Background/Aims: It has been described that cells in culture with very low oxidative metabolism possess a low CO₂ membrane permeability, P_{CO_2} , of ~ 0.01 cm/s. On the other hand, cardiomyocytes and mitochondria with extremely high rates of O₂ consumption exhibit very high CO₂ membrane permeabilities of 0.1 and 0.3 cm/s, respectively. To ascertain that this represents a systematic relationship, we determine here P_{CO_2} of hepatocytes, which exhibit an intermediate rate of O₂ consumption. **Methods:** We isolated intact hepatocytes with vitalities of ~ 70% from rat liver and measured their CO₂ permeability by the previously published mass spectrometric ¹⁸O exchange technique. **Results:** We find a P_{CO_2} of hepatocytes of 0.03 cm/s in the presence of FC5-208A and verapamil. FC5-208A was necessary to inhibit extracellular carbonic anhydrase, and verapamil was necessary to inhibit intracellular uptake of FC5-208A by the organic cation transporter OCT1 of hepatocytes. **Conclusion:** Rat hepatocytes with their intermediate rate of oxygen consumption also possess an intermediate CO₂ permeability. From pairs of data for five types of cells/organelles, we find an excellent positive linear correlation between P_{CO_2} and metabolic rate, suggesting an adaptation of P_{CO_2} to the rate of O₂ consumption.

Introduction

It has been demonstrated that the CO₂ permeability (P_{CO_2}) can vary between different cell types. Membranes from various cells and organelles have shown P_{CO_2} values varying between 0.001 cm/s for the apical membrane of colon epithelium [1] to 0.3 cm/s for the

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mitochondrial membrane [2]. These differences in P_{CO_2} have been shown to be associated with two main factors: the cholesterol content of the membrane [3] and the presence/absence of CO₂ channels in the membrane [3–5].

Another observation has been that cultured MDCK and tsA201 cells, with very low oxygen consumptions of ~12 nmol/s/ml [2], exhibit very low P_{CO_2} values of 0.017 and 0.007 cm/s, respectively [3], whereas heart cells with a maximal oxygen consumption of around 300 nmol/s/ml have a permeability of 0.1 cm/s, and mitochondria with an even higher maximal oxygen consumption of ~1000 nmol/s/ml have the highest CO₂ permeability ever measured for a biological membrane of 0.3 cm/s [2].

This latter observation raises the question if there is a positive relationship between P_{CO_2} and the metabolic rate, i.e. the rate of oxygen consumption rate and/or the about equal rate of CO₂ production, of a cell/organelle. To further explore this hypothesis, we decided to measure the CO₂ permeability of hepatocytes. These cells have an oxygen consumption in the middle range between MDCK cells and cardiomyocytes. Thus, these data were expected to allow us to substantiate or disprove the idea of a correlation between cell membrane P_{CO_2} and metabolic rate.

Materials and Methods

Cell isolation

Experiments were performed using 3 months old female Lewis rats. The animal experiments were approved by the local authority for animal experimentation. Female rats were preferred because they express carbonic anhydrase type II, which is sulphonamide-sensitive, in a greater proportion than males, which express mainly CA III (sulphonamide-insensitive) due to hormonal causes [6]. The protocol for isolation of the hepatocytes was adapted from the one proposed by Shen et al. [7].

One hour before the surgery, all buffers were immersed in a water bath at 45°C. Animals were anesthetized with isoflurane, the abdomen was cut open, and the caval vein was exposed and cannulated with an 18G cannula that was secured with a string. The perfusion was started with the HBSS buffer without Mg²⁺ and Ca²⁺ (Invitrogen, ThermoFisher Sci., Braunschweig, Germany), supplemented with MgCl₂ 0.9 mM, EDTA 0.5 mM, HEPES 25 mM at a flow rate of 10ml/min. After cannulation was confirmed successful, the portal vein was cut open and the flow increased to 25ml/min for 3min. Then the perfusion buffer was switched to the one containing: HBSS buffer with Mg²⁺ and Ca²⁺ (Invitrogen), 1.25U/mL of collagenase type 2 (Worthington, CellSystems Biotechnol., Troisdorf, Germany) and 25mM HEPES for another 6 minutes.

The liver was taken out and put into a cell culture dish with 20ml of ice-cold William's Medium E (Invitrogen) supplemented with 5% fetal bovine serum (Gibco, ThermoFisher Sci., Braunschweig, Germany) and 2mM L-Glutamine. The liver was disrupted gently using a forceps. The solution with the cells was passed through a 100 µm cell strainer and then medium was added to give a total volume of 40ml. Cells were then centrifuged at 60g for 3 min at 4°C, and supernatant was discarded. The cell pellet was treated with Red Blood Cell Lysis buffer (Roche, Merck KGaA, Darmstadt, Germany) in a proportion of 1 part pellet to 2 parts lysis buffer and incubated for 5min at room temperature, then centrifugation at 50g for 3min at 4°C was performed and the supernatant was discarded. The cells were then incubated in 25 ml of William's Medium E on ice for 5min, and subsequently 25 ml 90% Percoll (GE Healthcare Life Sci., Freiburg, Germany) in PBS was added. With this solution, another centrifugation step at 200g for 10min at 4°C was performed, and the pellet was washed with William's Medium E supplemented only with L-glutamine. Finally, the pellet was resuspended in 500 µl of this solution for further experiments. These cell suspensions had a cytotrit of 8·10⁶/ml on average. The cell suspension from each experiment was used without further dilution for mass spectrometric measurements of intact cells as well as of lysed cells.

Cell density was determined in the microscope using a Neubauer chamber. Cell viability was assessed by trypan blue dye exclusion. The diameter of the cells was determined microscopically, and for the calculation of intracellular volume and cell surface we assumed a spherical shape of the cells.

Mass spectrometric assay of cellular CO₂ permeability

A reaction chamber with a volume of 1.7 ml was used. It was attached to the high vacuum of the mass spectrometer via the previously published inlet system [1, 8, 9]. This chamber had a water-jacket that kept

the solutions at 37°C, and contained a stirrer that mixed the content continuously. The pH was adjusted to 7.4 and monitored with a pH electrode during the entire mass spectrometric measurement.

Solutions. CO₂ permeability experiments were performed using a buffer that contained 110 μM NaCl and 20 mM HEPES (with later addition of 25 mM sodium bicarbonate) with a pH of 7.4. Lysate experiments were performed using a buffer with 68 mM NaCl, 23.7 mM K₂SO₄ and 20 mM HEPES with a pH of 7.2. For each experiment with intact cells, 30 μl of cell suspension was added into the reaction chamber attached to the mass spectrometer, and experiments were ended by the addition of an excess of carbonic anhydrase.

Inhibitors

Organic cation transporter OCT1 inhibitor Verapamil (Sigma-Aldrich Chemie, Taufkirchen, Germany) was used in a final concentration of 20 μM, and extracellular carbonic anhydrase inhibitor FC5-208A [10] was applied at a final concentration of 5x10⁻⁵ M. Both inhibitors were used in all experiments with intact cells. To several experiments, the inhibitor of the aquaporin 1 (AQP1)- and Rhesus-associated glycoprotein (RhAG)-gas channels 4,4'-Diisothiocyanato-2,2'-stilbenedisulfonate (DIDS; Sigma-Aldrich Chemie, Taufkirchen, Germany) was added in a final concentration of 1x10⁻⁴ M [4, 5].

Determination of P_{CO₂} and P_{HCO₃⁻}

The determination of the two parameters uses the mass spectrometric observation of the disappearance of ¹⁸O-labelled CO₂, as described in detail by Endeward and Gros [1]. A typical recording can be seen below in Fig. 2D.

The principle of this technique is as follows. A reaction chamber is filled with a solution of 110 mM NaCl with 20 mM HEPES to which 25 mM HCO₃⁻ is added at pH 7.4, where the HCO₃⁻ has been labelled with ¹⁸O by 1%. The fluid in the chamber is connected to the high vacuum of a mass spectrometer via a gas-permeable 25 μm thick Teflon membrane supported by a sintered glass disc. Thus, the mass spectrometer can continuously follow the time course of the partial pressure of C¹⁸O¹⁶O inside the reaction chamber. The very first phase seen in the recording of Fig. 2D (and all other recordings in this figure) represents the spontaneous decay of [C¹⁸O¹⁶O] in the chamber, which is due to a slow loss of ¹⁸O from the CO₂-HCO₃⁻ pool into the much larger water pool. This exchange of ¹⁸O is mediated by the reaction HCO₃⁻ + H⁺ ↔ CO₂ + H₂O, where each reaction step from left to right has a chance of 1/3 of transferring an ¹⁸O from the singly labelled bicarbonate into water. Of course, this exchange of ¹⁸O is greatly accelerated, when the dehydration-hydration reaction is accelerated by carbonic anhydrase, such as in Fig. 2D by the addition of CA-containing cells. The two phases seen in Fig. 2D to follow upon the addition of cells, represent the rapid influx of C¹⁸O¹⁶O into the cells (first faster phase) and the slower influx of labelled HCO₃⁻ (second slower phase). Analysis of the two phases on the basis of the complete physico-chemical description of the entire reaction and diffusion process [1] can therefore provide estimates of P_{CO₂} and P_{HCO₃⁻} of the cell membrane.

Application of the ¹⁸O exchange technique to assess the permeability of the membrane for CA inhibitors

The type of experiments shown in Fig. 2 A,B and C allows one also to assess whether the membrane studied is permeable or impermeable to a carbonic anhydrase inhibitor. This is important, because determination of P_{CO₂} and P_{HCO₃⁻} from mass spectrometric measurements requires that a) the cells studied contain high CA activity, but also b) that the extracellular solution is free of CA activity. Because hepatocytes possess a membrane-bound extracellular CA, the present experiments necessitate the application of an extracellular CA inhibitor such as the FC5-208A used here. Whether this inhibitor is truly restricted to the extracellular space can be tested as illustrated in the theoretical curves of Fig. 1. The ¹⁸O exchange measurement simulated in Fig. 1 is calculated for the presence of high intracellular CA activity in combination with moderate extracellular CA activity. After the very first phase in Fig. 1, the addition of cells is simulated, which is followed by a biphasic time course of the C¹⁸O¹⁶O signal. Then, in Fig. 1A addition of a membrane-permeable CA inhibitor is simulated, and in Fig. 1B addition of a membrane-impermeable extracellular inhibitor is simulated. It is apparent that the permeable (= extra- plus intracellular) inhibitor causes an upward deflection of the mass spectrometric signal, whereas the impermeable (= extracellular) inhibitor causes a downward deflection. The upward deflection is due to intracellular CA inhibition, the downward deflection due to the purely extracellular CA inhibition. Thus, this maneuver allows one to test in a simple fashion whether a CA inhibitor enters the intracellular space or not. This test was applied to the inhibitor FC5-208A, and hepatocytes were seen to take up the inhibitor, as explained below.

Performing ¹⁸O exchange experiments with hepatocytes

For control experiments, 100 µl of cell suspension was incubated with verapamil for 5 min in order to inhibit the OCT1, because the latter was found to transport the extracellular carbonic anhydrase inhibitor FC5-208A into the cell interior (see below). During this time, the reaction chamber was filled with the buffer, 25 mM labelled HCO₃⁻, verapamil and FC5-208A were added. Then, the first part of the mass spectrometric recording was taken for a little less than 1 min, showing the spontaneous decay of C¹⁸O¹⁶O. After this first part of the recording, 30 µl of cell suspension was added and led to an acceleration of the decay of C¹⁸O¹⁶O, which occurred in two phases, a first fast and a second slower phase. After this biphasic time course was recorded for a sufficiently long time, the experiment was ended by the addition of an excess of soluble carbonic anhydrase in order to establish the final isotopic equilibrium. To study the effect of DIDS on P_{CO₂} and P_{HCO₃⁻}, the above procedure was repeated identically, except that 10⁻⁴ M DIDS was added to the 100 µl cell suspension at the beginning of the 5min incubation time with verapamil. In addition, the same DIDS concentration was present in the buffer filled into the reaction chamber before cells were added.

Determination of the intracellular carbonic anhydrase activity (CAi)

Cell suspensions as described above were treated with Triton at a final concentration of 0.1% and then frozen. For the acquisition of a mass spectrometric recording, the suspensions were thawed and 30µl of the lysed cells was added to the chamber containing the above lysate buffer, 11mM labeled HCO₃⁻ and Triton 0.1%. Addition of the lysates caused a monophasic acceleration of the decay of the C¹⁸O¹⁶O signal. The experiment was again ended with the addition of excess carbonic anhydrase. The record was analyzed in MATLAB (The MathWorks Inc., Paderborn, Germany) to obtain the carbonic anhydrase activity from the increase in slope seen after addition of the lysate. Activity was then standardized by the cell density and volume estimated before lysis for each sample to obtain the intracellular CA activity of the cells. This quantity was necessary to calculate P_{CO₂} and P_{HCO₃⁻} from the mass spectrometric records of intact hepatocytes [1].

Statistical Analysis

Data are presented as mean and ±SD. The Student's t-test for paired samples was performed to compare n pairs of permeabilities with and without DIDS each obtained from the same rat.

Results

Inhibition of extracellular CA of hepatocytes

The mass spectrometric ¹⁸O-exchange technique used here requires that the extracellular membrane-bound CA of hepatocytes (CAIV) [11, 12] is completely inhibited. With other cells, we have in the past achieved this [4, 13] by the addition of the extracellular CA inhibitor FC5-208A as introduced by Perut et al. [10]. After performing the first experiments to establish the final protocol for the mass spectrometric experiments, a discrepancy in P_{CO₂} between pure cell suspensions and cells incubated with the extracellular CA inhibitor FC5-208A was observed. To understand the reason for this observation, FC5-208A was added into the reaction chamber during the run of a control experiment, and it was observed that the record answered with an upward “bump” (Fig. 2A). The theory of the ¹⁸O-exchange process predicts, as shown in Fig. 1B, that a pure inhibition of extracellular CA should cause a downward deflection of the recorded curve. In contrast, it is known that inhibition of intracellular CA will cause an upward deflection and a bump like that seen in in Fig. 1A and Fig. 2A. That this interpretation is correct, is confirmed by the experiment of Fig. 2B, in which during the course of a control experiment the CA inhibitor ethoxzolamide was added, which is well-known to be highly membrane-permeable and thus to be an effective inhibitor of intracellular CA. The clear-cut conclusion is that FC5-208A in the case of hepatocytes does not remain in the extracellular space but enters the cells and inhibits intracellular CA. The theory of the ¹⁸O-exchange process presupposes that extracellular CA is absent or inhibited while intracellular CA is unaffected. Full or partial inhibition of the intracellular CA will seriously distort the mass spectrometric recording and make determination of P_{CO₂} and P_{HCO₃⁻} impossible. Thus, it was necessary to find a way of inhibiting extracellular CA without inhibiting intracellular CA.

Fig. 1. Simulation of mass spectrometric measurements of ¹⁸O exchange illustrating the effects of an extra- and intracellularly active carbonic anhydrase inhibitor (A) and of a purely extracellularly active CA inhibitor (B). The simulation was performed using numerical solutions of the system of equations as previously described [1].

Fig. 2. Original mass spectrometric recordings of liver cells under different conditions. A, recording from a hepatocyte cell suspension with later addition of extracellular CA inhibitor FC5-208A (2nd arrow) during the experimental run. B, recording of hepatocytes with addition of ethoxzolamide during the experimental run (2nd arrow); ethoxzolamide is a highly membrane-permeable CA inhibitor. C, experimental run with hepatocytes which had been preincubated with 20μM verapamil for 5min. The same concentration of verapamil was present in the reaction chamber before the addition of cells. The later addition of the extracellular CA inhibitor FC5-208A (2nd arrow) caused practically no upward bump. D, a recording performed with a verapamil-preincubated cell suspension. Again, verapamil + extracellular CA inhibitor had been added into the chamber prior to the addition of the cells. Fig. 1D represents the final protocol used for all experiments of this study.

The FC5-208A is an organic cation, which with most cells remains extracellular due to its positive charge [10]. However, it is known that rat hepatocytes possess OCT1 [14],

an organic cation transporter that might be able to transport FC5-208A into the cell interior. To examine this possibility, we tested successfully some OCT1 inhibitors and found verapamil the most suitable one. We used verapamil in a concentration of 32x the IC₅₀ for the OCT1 substrate metformin [15], 14 times the IC₅₀ for serotonin [16], and 6x the IC₅₀ for tetraethylammonium TEA [17], to achieve a nearly complete inhibition of OCT1-mediated transport. After 5 min incubation of the cell suspensions with verapamil, we repeated the addition of the FC5-208A during the course of the same mass spectrometric experiment as performed in Fig. 2A. We now observed that the upward “bump” had disappeared, which shows that no longer inhibition of the intracellular CA occurred (Fig. 2C). After these findings, verapamil was included in all experiments in order to be able to use the FC5-208A to inhibit extracellular CA without affecting intracellular CA. This made it possible to determine the P_{CO₂} of hepatocytes from the usual mass spectrometric recordings of cell suspensions, such as shown in Fig. 2D.

Carbonic anhydrase activity of isolated hepatocytes

The freshly prepared hepatocytes had an average vitality of 70%. In order to assess whether only vital cells, but not non-vital cells, contribute to the mass spectrometric signal, it is important to know whether the fraction of non-vital cells has still carbonic anhydrase. Generally, a mass spectrometric signal is generated only by cells possessing intracellular CA activity [1]. In a previous study on cardiomyocytes it has been possible to use a CA-staining that stains all CA isoforms, the fluorescence staining with dansylsulfonamide (DNSA) [13, 18]. This showed that non-vital cardiomyocytes no longer possess CA activity and thus do not contribute to the mass spectrometric signal. In the case of hepatocytes, we were not able to use DNSA staining because of the very high auto-fluorescence of hepatocytes [19]. For hepatocytes, we use an alternative approach. In Fig. 3 data points from 10 hepatocyte preparations are plotted. For each preparation, we use a) the cell density in the final cell suspension, b) the vitality of each cell preparation, and c) the CA activity measured in the lysed but undiluted cell suspension. The figure shows the lysate CA activity divided by the cell density on the ordinate, and % vitality on the abscissa. It is apparent that lysate CA activity normalized for cell density correlates reasonably well with cell vitality (slope of the linear regression $0.68 \cdot 10^{-7}$ ml, $r = 0.64$; $P = 0.046$). The regression shows that normalized lysate CA activity decreases linearly and significantly with decreasing vitality. At the same time, the Y-axis intercept of $1.38 \cdot 10^{-7}$ is not significantly different from zero ($P = 0.54$). Thus, these experimental data are in agreement with a proportional relation between normalized lysate activity and vitality. This implies that only vital cells possess CA activity and non-vital cells neither contribute significantly to CA activity nor to the mass spectrometric signal.

Rat hepatocytes possess an intracellular CA activity of 1,900 (SD ± 590 , $n=8$). This number is calculated by assuming that all lysate CA activity is due vital cells only. This calculation also presumes that extracellular CA bound to the plasma membrane makes only a very small contribution to total cellular CA activity as measured in the lysate. This is usually justified [13]. There is, however, a minor contribution of mitochondrial CA [12].

Fig. 3. Correlation between lysate CA activity per cell density and cell vitality for rat hepatocyte cell suspensions. Linear regression $r = 0.64$, $P = 0.046$.

Fig. 4. P_{CO₂} of rat hepatocytes and the effect of DIDS. * indicates statistical significance ($T=3.098$, $df=7$, $p=0.017$).

Hepatocyte CO₂ and HCO₃⁻ permeability and morphological parameters

Estimation of P_{CO₂} and P_{HCO₃⁻} from the mass spectrometric records requires – besides intracellular CA activity – knowledge of cell volume and cellular surface-to-volume ratio. The average cell diameter was determined microscopically to be 20 μm (SD ±3μm). Based on the assumption of a sphere, the volume is calculated to be 4,546 μm³ (±1,966 μm³), the cell surface 1,299 μm² (±383μm²), and the surface-to-volume ratio 3057 cm⁻¹ (±500cm⁻¹). These morphological values were calculated on the basis of microscopy of 112 cells, they are concordant with the literature [20].

All CO₂ and HCO₃⁻ permeabilities were measured in the presence of the extracellular CA inhibitor in the chamber. With this, we found P_{CO₂} to be 0.032 cm/s (SD ±0.01, n=8) under control conditions. P_{CO₂} was significantly diminished to 0.021 cm/s (SD ±0.006, n=8; P=0.017) in the presence of 10⁻⁴ M DIDS (Fig. 4). The HCO₃⁻ permeability also decreased in the presence of DIDS from 7.2·10⁻⁴ cm/s (SD ±5.0·10⁻⁴) to 3.7·10⁻⁴ cm/s (SD ±3.8·10⁻⁴), although this difference was not significant.

Discussion

CA activity of hepatocytes

Hepatocytes present several membrane-bound and cytoplasmic carbonic anhydrases. CA IV is the membrane-bound form present on the plasma membrane (i.e. extracellular) and the endoplasmic reticulum (i.e. intracellular), and CA II and CA III are the cytoplasmic isoforms [11, 12]. There is also the mitochondrial isoform CAV, but its contribution to total cellular CA activity is minor [12].

We report here a carbonic anhydrase activity for hepatocytes of 1,900 at 37°C, indicating that the rate of CO₂ hydration in a hepatocyte is accelerated over the uncatalyzed rate by a factor of 1,900+1. This activity includes all cytosolic and all membrane-bound forms of CA. If we compare this rate to the one published by Ono et al. [11] and assume that the hepatocytes have an overall protein concentration of 200 mg/ml, we obtain from their data a hepatocyte CA activity of 1,400, in good agreement with the present value.

Bicarbonate permeability of hepatocytes

In rat hepatocytes several membrane proteins capable of bicarbonate transport have been found: SAT-1 [21, 22], NBC4 [23], AE2 [24, 25], and OATP [26]. In our experiments, because of the conditions that our cells are exposed to inside the chamber, it is possible that the DIDS-sensitive anion exchanger 2, AE2, will have the dominant role in bicarbonate transport. This might explain why, in the presence of DIDS, we were able to see a reduction of the bicarbonate permeability by around 30%, although not significant.

CO₂ permeability of hepatocytes

It can be considered established now that the CO₂ permeability of biological membranes depends on two major factors: the membrane cholesterol content and the presence/absence of CO₂ channels in the membrane [3, 27–29]. In the case of hepatocytes we find that the presence of DIDS lowers the CO₂ permeability by one third, from 0.32 cm/s to 0.21 cm/s. This latter fact shows that CO₂ channels likely contribute to the total CO₂ permeability of hepatocytes.

DIDS is capable of inhibiting CO₂ transport across AQP1 [3, 4] and across Rhesus-associated glycoprotein, RhAG [5,

Table 1. Oxygen consumption and P_{CO₂} of different cell types and mitochondria. a-[2], b-converted from Del Castillo et al. [43], c- converted from [44], d- converted from Schmidt & Thews [45], e-[3], f-[1], g-[14]

Type of cell/organelle	Rate of Oxygen Consumption	P _{CO₂} (cm/s)
	nmol*s ⁻¹ *ml ⁻¹	
MDCK	12 ^a	0.017 ^e
Basolateral membrane PCE	54.7 ^b	0.022 ^f
Rat Hepatocytes	69.3 ^c	0.03
Rat Cardiomyocytes	297 ^d	0.1 ^g
Mitochondria	1000 ^a	>0.3 ^a

30]. Rat hepatocytes express AQP0, AQP8, AQP9 and AQP11 [25, 31, 32]. From them, AQP0 and AQP9 are known CO₂ channels [33]. AQP0 is mainly intracellular but AQP9 is expressed in the plasma membrane of hepatocytes [32] and therefore is expected to participate in CO₂ transport across the hepatocyte membrane. A second candidate for CO₂ transport are the non-erythroid forms of Rhesus proteins, RhBG and RhCG [34]. These proteins have been described in mouse liver [35, 36] and may play a role in ammonia uptake and pH regulation of hepatocytes [37]. In both cases (AQP9 and non-erythroid Rh proteins), no information is available regarding the DIDS sensitivity of their pathways for CO₂. Therefore, strictly speaking it remains speculative whether the DIDS effect we observe is due to one or both of these CO₂ channels (or to an unknown other one).

On the other hand, the most important conclusion from this measurement is that P_{CO₂} also in hepatocytes seems essentially determined by the cholesterol content of the membrane. Hepatocytes have a cholesterol content in their plasma membrane of between 0.32 to 0.41 mol cholesterol/mol total lipids [38–40]. According to Itel *et al* [3], a phospholipid membrane with this amount of cholesterol should, in the absence of CO₂ channels, possess a permeability between 0.02 and 0.03 cm/s, which is entirely consistent with the present results, with and without DIDS. We conclude that the P_{CO₂} of hepatocytes is mainly defined by the cholesterol content of the membrane, and is increased only to a minor extent by the contribution of CO₂ channels. This is especially apparent, when we compare this situation with the case of human erythrocytes, in which the CO₂ channels AQP1 and RhAG increase the “intrinsic” membrane permeability from 0.015 cm/s by a factor of 10 to 0.15 cm/s [4, 5, 30]. Also in view of this latter P_{CO₂} value, it should be noted that the P_{CO₂} of hepatocytes is comparatively low when compared to some other biological membranes (see Table 1 below). Although some metabolic pathways in liver cells consume bicarbonate [41, 42], it is likely that this requirement can easily be satisfied by the endogenous CO₂ production of the liver cell. Thus, there is no need for CO₂ uptake into the liver cell from blood, and P_{CO₂} of liver cells must not cope with CO₂ fluxes exceeding those necessary for the release of the CO₂ generated by mitochondrial respiration.

CO₂ permeability and aerobic metabolism

The present work was prompted by the hypothesis that the CO₂ permeability of most cells and organelles correlates with their rate of aerobic metabolism. As O₂ consumption is associated with an approximately equal CO₂ production, a cell membrane P_{CO₂} that is adapted to the metabolic rate would contribute to CO₂ homeostasis in the cell, avoiding for example cellular respiratory acidosis.

To prove or disprove this idea, we compiled published data of oxygen consumption of various cell types and the P_{CO₂} values for these cells together with the present P_{CO₂} of hepatocytes (Table 1). As apparent from Table 1, the present hepatocyte data were crucial for this purpose, because they represent the middle range between very low and very high P_{CO₂} values as well as rates of oxygen consumption. The only other value in this middle range is that for the basolateral membrane of proximal colon epithelium. The interpretation of this value, however, is complicated it because it refers to a polarized cell, whose apical membrane has a drastically different, extremely low P_{CO₂} [1]. Nevertheless, we have included it in Table 1, because the basolateral membrane is the one participating in CO₂ exchange with

Fig. 5. Linear regression between P_{CO₂} and O₂ consumption for (from right to left) mitochondria, cardiomyocytes, hepatocytes, basolateral membrane of colon epithelium, and MDCK cells. $r=0.9996$, $p<0.0001$. The insert shows the enlarged left-hand portion of the regression curve.

the body. The lowest P_{CO_2} in Table 1 is exhibited by a cell line in culture, MDCK cells, which show the lowest oxygen consumption of the table. The other extreme is represented in Table 1 by cardiomyocytes, which *in vivo* constitute the cells with the highest maximal oxygen consumption in the body, and by mitochondria with an even higher specific rate of oxygen consumption.

Fig. 5 shows a graphical representation of the numbers of Table 1. It illustrates that there is an excellent positive linear correlation between P_{CO_2} and specific oxygen consumption ($r = 0.9996$). This holds also for the lower values of P_{CO_2} and oxygen consumption, as indicated by the enlargement of the left-hand part of the figure shown in the insert. Although the Y-intercept is 0.01 cm/s and not quite zero, we conclude that indeed cellular P_{CO_2} and specific oxygen consumption are closely and almost proportionally related to each other. This would be compatible with the idea of an excellent adaptation of P_{CO_2} to specific oxygen consumption.

What can be the physiological purpose of raising P_{CO_2} with increasing specific oxygen consumption? Endeward et al. [46] have in a simple approach analyzed the role that the diffusion resistance of the plasma membrane can play for cellular CO₂ elimination under steady state conditions. Their model uses an intracellular diffusion path of 7 μm and a membrane of variable P_{CO_2} , both together constituting the two resistances to be overcome by the CO₂ released from the mitochondrion. For an arbitrarily chosen but reasonable CO₂ partial pressure difference between the site of CO₂ production and the extracellular space ($\Delta p\text{CO}_2$) of 0.35 mmHg, they find that for the maximal rate of O₂ consumption - and CO₂ production - of a cardiomyocyte of $\sim 300 \text{ nmol} \cdot \text{s}^{-1} \cdot \text{ml}^{-1}$, P_{CO_2} values down to 0.2 cm/s exert no limiting effect on cellular CO₂ release. The much lower P_{CO_2} of 0.01 cm/s, on the other hand, would decrease CO₂ release to about 1/4 of CO₂ production. This implies that the low P_{CO_2} will force the cell to increase its CO₂ partial pressure in order to maintain a sufficient rate of CO₂ release. With this model and an identical intra-extracellular $\Delta p\text{CO}_2$, a cell with a three times lower P_{CO_2} such as the hepatocyte can be shown to release its CO₂ without diffusion limitation due to its three times lower rate of CO₂ production. The same holds for MDCK cells, whose P_{CO_2} is 6 times lower than that of cardiomyocytes but whose oxygen consumption is 25 times lower. Thus, the hypothesis seems possible that in general cellular P_{CO_2} increases with oxygen consumption in order to avoid otherwise necessary increases in intracellular CO₂ partial pressure. While this hypothesis will require further experimental substantiation, we have here for the first time established an amazingly strict empirical relationship between P_{CO_2} and oxygen consumption.

In conclusion, the variability of the CO₂ permeability of many cells is due to a strong correlation between P_{CO_2} and rate of CO₂ production. The value of the CO₂ permeability in all these cases is largely determined by the cholesterol content of the cell/organelle membrane. Red blood cells seem to constitute an exception by having no oxidative metabolism in combination with a high CO₂ permeability, the latter being almost entirely due to the presence of a high density of CO₂ channels [4, 5, 30]. Very likely, this exception relates to the special function of red cells in gas transport.

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Disclosure Statement

No conflict of interests exists.

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