Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 62 (2004) 153–158
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Oxygen sensitivity of red cell membrane transporters revisited

Clare Drew[a], Vicky Ball[a], Hannah Robinson[a], J. Clive Ellory[a], John S. Gibson[b,*]

[a] University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK
[b] Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK

Received 6 June 2003; received in revised form 23 July 2003; accepted 25 July 2003

Abstract

In this paper, we provide an update on O_2-dependent membrane transport in red cells. O_2-sensitive membrane transport was compared in nucleated (chicken) and enucleated (human) red cells, to investigate effects on organic (glucose transporter [GLUT]) and inorganic (K^+-Cl^- cotransporter [KCC]/$Na^+-K^+-2Cl^-$ cotransporter [NKCC]) transporters, to study the response of so-called ''housekeeping'' transporters (Na^+/K^+ pump and anion exchanger [AE]) and, finally, to compare O_2 sensitivity in normal human red cells with those from sickle cell patients. The Na^+/K^+ pump showed no change in activity between oxygenated and deoxygenated cells in any of the samples. KCC in normal human red cells had the greatest O_2 sensitivity, being stimulated some 20-fold on oxygenation. It was more modestly stimulated by O_2 in chicken red cells and HbS cells. By contrast, NKCC was stimulated by deoxygenation in all cases. GLUT showed little response to O_2 tension, other than a small stimulation in deoxygenated chicken red cells. Finally, AE1 was stimulated by oxygenation in HbA cells, but this stimulation by O_2 was absent in HbS cells and pink ghosts prepared from HbA cells. The significance of these findings is discussed.

Keywords: O_2; GLUT; KCC; NKCC; Na^+/K^+ pump; AE

1. Introduction

Efficient exchange of blood gases is a major function of the circulatory system. Movement of O_2 and CO_2 between blood and pulmonary alveoli or peripheral tissues is dependent largely on passive diffusion, and hence requires a concentration difference. Thus, the partial pressure of these gases varies through the circulation, and this is particularly noticeable for that of the less soluble gas, O_2. Red cells are exposed to O_2 tensions, which in higher vertebrates may vary about 100-fold, from about 100 mm Hg on the arterial side of the circulation to <5 in metabolically active regions. In some lower vertebrates, even greater differences are seen, with, for example, very high O_2 tension in the choroid rete of fish (such as the trout) required to supply the O_2 demands of the retina [1].

As well as providing the concentration gradient for loading and unloading of O_2, onto the red cell haemoglobin (Hb), variations in O_2 tension also represent a specific signal capable of regulating the activity of many red cell membrane transport proteins. Whilst this is not a new observation—O_2-sensitive cation transport in avian red cells has been known for about half a century [2,3]—over the last few years, the significance of O_2 as a membrane transport controller, important both physiologically and pathologically, has become more apparent [4–6]. It is now recognised that the interaction between O_2 and other stimuli can be a critical determinant of red cell transporter activity [7].

The response to O_2 differs across species and transporters, whilst in certain haemoglobinopathies, for example in red cells from sickle cell patients, abnormal responses to O_2 are observed [8,9]. A systematic study, however, is in many cases lacking. In this paper, we provide an update on O_2-dependent membrane transport in red cells. Our rational was to compare O_2-sensitive transport in nucleated (chicken) and enucleated (human) red cells, effects on organic (glucose transporter [GLUT]) and inorganic (K^+-Cl^- cotransporter [KCC]/Na^+-

Abbreviations: AE, anion exchanger; ATP, adenosine triphosphate; DIDS, 4,4′ -diisothiocyano stilbene-2,2′ -disulfonic acid; DOG, 2-deoxy-glucose; EDTA, ethylene diamine tetra-acetic acid; EGTA, ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid; GLUT, glucose transporter; Hb, haemoglobin; HEPES, *N*-[hydroxyethyl]piperazine-*N*′ -[2-ethanesulphonic acid]; KCC, K^+-Cl^- cotransporter; MOPS, 3-(*N*-morpholino)propane sulphonic acid; NHE, Na^+/H^+ exchanger; NKCC, $Na^+-K^+-2Cl^-$ cotransporter; PIPES, piperazine-*N*,*N*′ -bis[2-ethanesulphonic acid].

* Corresponding author. Tel.: +44-1223-337638; fax: +44-1223-337610.
E-mail address: jsg1001@cam.ac.uk (J.S. Gibson).

1567-5394/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2003.07.003

K^+–$2Cl^-$ cotransporter [NKCC]) transporters, the response of so-called ''housekeeping'' transporters (Na^+/K^+ pump and anion exchanger [AE]), and finally to compare transport in normal human red cells with those from sickle cell patients.

2. Material and methods

2.1. Blood samples

Blood was obtained from healthy donors (HbAA) and homozygous sickle cell anaemia (HbSS) patients who had not been transfused for at least 6 months—all from informed volunteers and with ethical consent. HbA-containing red cells are referred to as HbA cells; those containing HbS, as HbS cells. Chicken blood was obtained from Harlan-Sera Lab (Loughborough, UK). Blood samples were centrifuged (5 min, 850 × *g*, room temperature) and the buffy coat and plasma aspirated to remove platelets and white blood cells, red cells were then washed a further three times with the appropriate saline. Samples were stored on ice until required (within 36 h).

2.2. Chemicals and salines

Salts, creatinine kinase, bumetanide, 2-deoxyglucose (DOG), 4,4′ -diisothiocyano stilbene-2,2′ -disulfonic acid (DIDS), ethylene diamine tetra-acetic acid (EDTA), ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′ ,*N*′ -tetra-acetic acid (EGTA), glucose, *N*-[hydroxyethyl]piperazine-*N*′ -[2-ethanesulphonic acid] (HEPES), 3-(*N*-morpholino)propane sulphonic acid (MOPS), piperazine-*N*,*N*′ -bis[2-ethanesulphonic acid] (PIPES) and ouabain were obtained from Sigma (Poole, Dorset, UK). $^{86}Rb^+$ and ^{3}H-2-deoxyglucose were purchased from NEN Life Sciences (Stevenage, UK); $^{35}SO_4^{2-}$ from Amersham Pharmacia Biotech UK (Bucks, UK). Saline for use with human blood contained (mM): NaCl (150), MOPS (10), glucose (5), 290 mosM kg^{-1}; and for chickens, NaCl (155), HEPES (10), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1), glucose (5), 320 mosM kg^{-1}; both pH 7.4 at 37 °C. For Cl^- dependency, Cl^- salts were replaced by NO_3^- ones. For SO_4^{2-} fluxes, sulphate saline contained (mM) Na_2SO_4 (107), MOPS (10), glucose (5) and sucrose saline contained sucrose (300), MOPS (10); both pH 7.4 at 37 °C. For ghost preparation, lysing solution (LS) contained (mM) EDTA (0.1), PIPES (15), pH 6.5 at 0 °C, and resealing solution (RS) NaCl (10), KCl (140), MOPS (10), $MgCl_2$ (0.15), EGTA (0.1), Na phosphocreatine (5), K_3ATP (3) and creatine kinase 10 units ml^{-1}, pH 7.4 at 37 °C.

2.3. Preparation of pink ghosts

Pink ghosts were made from normal (HbA) human red cells by adding cooled packed red cells to LS at 0 °C, leaving for 5 min, before centrifugation (5 min, 20,000 g, 0 °C) and removal of the supernatant. Cooled RS (5 × initial cell volume, to give a ghost dilution of 1 in 5) was added to the pellet, and then left on ice for 10 min, before incubating for 1 h at 37 °C to allow resealing. Ghosts were then centrifuged (5 min, 40,000 × *g*, room temperature), and washed three times in saline by successive centrifugations (same conditions) and resuspensions.

2.4. Tonometry

Red cell or ghost samples were placed at 20% cytotocrit in tonometers (Eschweiler and Kiel, Germany). They were equilibrated with either air or N_2 for 15 min, fully humidified at 37 °C. Samples were then transferred to test tubes (pre-equilibrated at the same O_2 tension and continually gassed during the experiment) for measurement of influx or efflux.

2.5. Transporter activity

We chose to compare the O_2 sensitivity of the glucose transporter GLUT, the NKCC, the KCC, the Na^+/K^+ pump and the AE1.

2.5.1. K^+ influx for KCC, NKCC and Na^+/K^+ pump activities

These were assessed using $^{86}Rb^+$ influx (added in 150 mM K^+ to give a final [K^+] of 7.5 mM), as a congener for K^+, following the method of Dunham and Ellory [10]. Cl^- dependence of K^+ influx (in the presence of bumetanide, 10 μM) was used to assess KCC activity, bumetanide (10 μM)-sensitive K^+ influx for NKCC and ouabain (100 μM)-sensitive influx for Na^+/K^+ pump. Bumetanide (10 μM) and ouabain (100 μM) were present in all experiments, except for NKCC and pump assays. Haematocrit or ghost concentration was measured by the Drabkins method (see Ref. [10]) or in microhaematocrit tubes. K^+ and DOG influxes are expressed as mmol (l cells h)$^{-1}$ or mmol (l ghosts h)$^{-1}$. Experiments were carried out at 37 °C.

2.5.2. Deoxyglucose influx for GLUT activity

Glucose influx was assessed by measuring the uptake of ^{3}H-2-deoxyglucose in glucose-free saline containing 5 mM DOG, at room temperature (22 °C). Cells were then washed free of unincorporated radioisotope (Dunham and Ellory, 1981).

2.5.3. SO_4^{2-} efflux for AE activity

Red cells were washed three times in sulphate saline with 30 min incubations at 37 °C between each wash. Cells were then placed in sulphate/sucrose solution (10-fold dilution of sulphate saline into sucrose saline) and incubated for 1 h with $^{35}SO_4^{2-}$. Cells were washed free of unincorporated $^{35}SO_4^{2-}$ by four washes (10,000 × *g*, 10 s) in ice-cold sulphate saline. Samples were then placed in tonometers for equilibration at the appropriate O_2 tension. Efflux was measured by removing serial samples (200 μl), pelletting the

red cells (10,000 × *g*, 10 s) and counting the supernatant. A final sample was taken, lysed and deproteinated to give the total initial intracellular counts. For $^{35}SO_4^{2-}$ in the supernatant, efflux follows the equation: $(C_\infty - C_t)=(C_\infty - C_0)e^{-kt}$, where C_∞ = total initial intracellular counts radioactivity in cells at time 0, C_t = radioactivity in supernatant at time t, C_0 = sample at time 0, k = rate constant and t = time. The slope of $\ln[(C_\infty - C_t)/(C_\infty - C_0)]$ vs. t was used to estimate $-k$. Experiments were carried out at room temperature (22 °C). Control experiments showed that this SO_4^{2-} efflux was abrogated by the stilbene derivative, DIDS (5 μM), consistent with mediation via AE1 [11].

2.6. Statistics

Values are given as means ± S.E.M. for n observations, where *n* refers to samples from different animals or individuals.

3. Results and discussion

Oxygen sensitivity of the five transporters (GLUT, NKCC, KCC, Na^+/K^+ pump and AE) is presented in Table 1 (for chicken red cells), Table 2 (for HbA cells) and Table 3 (for HbS cells), and in Fig. 1 (Na^+/K^+ pump in HbA cells), Fig. 2 (KCC in chicken red cells and HbA cells) and Fig. 3 (AE1 in HbA cells).

3.1. Main findings

To summarise, the Na^+/K^+ pump showed no change in activity between oxygenated and deoxygenated cells in any of the samples (see Fig. 1). KCC in normal human red cells had the greatest O_2 sensitivity, being stimulated some 20-fold on oxygenation (Fig. 2). It was more modestly stimulated by O_2 in chicken red cells (Fig. 2) and HbS cells (although in the latter, the analysis is complicated by the biphasic response of KCC activity to O_2 tension—see Ref. [8]). By contrast, NKCC was stimulated by deoxygenation in all cases. GLUT showed little response to O_2 tension, other than a small stimulation in deoxygenated chicken red cells. Finally, AE1 was stimulated by oxygenation in HbA cells (Fig. 3), but this stimulation by O_2 was absent in HbS cells and pink ghosts prepared from HbA cells.

Table 1
Summary of O_2 sensitivity of membrane transporters in chicken red cells

	Flux in air	Flux in N_2	Air/N_2	N_2/air	O_2 dependence
GLUT-1	2.66 ± 0.95	3.67 ± 1.39	0.7 ± 0.0	1.4 ± 0.0	0
NKCC	0.72 ± 0.12	3.54 ± 1.20	0.2 ± 0.1	5.0 ± 1.8	−
KCC	1.08 ± 0.18	0.64 ± 0.22	1.7 ± 0.6	0.7 ± 0.3	+
Na^+ pump	4.06 ± 1.20	4.34 ± 1.14	0.9 ± 0.5	1.1 ± 0.6	0

Fluxes were determined in fully oxygenated or fully deoxygenated red cells and given in mmol (l cells h)$^{-1}$, and as means ± S.E.M. (all n = 3 or 4, except AE1 in HbA and HbS cells, where n = 7 and 6, respectively). GLUT-1 was defined as deoxyglucose influx, NKCC as bumetanide (10 μM)-sensitive K^+ influx, KCC as Cl^--dependent K^+ influx (in presence of bumetanide, 10 μM) and Na^+ pump as ouabain (100 μM)-sensitive K^+ influx. Air/nitrogen and nitrogen/air give the ratio of fluxes determined in oxygenated cells/deoxygenated red cells, and vice versa. Transporters are ascribed O_2 dependence if ratio >1.5 or <0.7; + means stimulated by oxygenation, − stimulated by deoxygenation, 0 O_2 insensitive. Abbreviations: GLUT-1 = glucose transporter isoform 1, NKCC = Na^+–K^+–2Cl^- cotransporter, KCC = K^+–Cl^- cotransporter, Na^+ pump = Na^+/K^+ pump.

Table 2
Summary of O_2 sensitivity of membrane transporters in normal human red cells (HbA cells)

	Flux in air	Flux in N_2	Air/N_2	N_2/air	O_2 dependence
GLUT-1	11.43 ± 1.86	10.80 ± 1.43	1.1 ± 0.0	0.9 ± 0.0	0
NKCC	0.36 ± 0.01	0.54 ± 0.06	0.6 ± 0.1	1.6 ± 0.2	−
KCC	0.36 ± 0.14	0.02 ± 0.01	20.6 ± 7.4	0.1 ± 0.0	+
Na^+ pump	1.68 ± 0.38	1.68 ± 0.32	1.0 ± 0.1	1.0 ± 0.1	0
AE1 cell	0.38 ± 0.02	0.25 ± 0.02	1.5 ± 0.1	0.7 ± 0.0	+
AE1 ghost	0.30 ± 0.03	0.27 ± 0.02	1.1 ± 0.1	0.9 ± 0.1	0

See Table 1 legend for details. For AE1, fluxes are given as the rate constant, k (h^{-1}), for SO_4^{2-} efflux. Abbreviations: AE1 cells indicates intact HbA cells; AE1 ghosts refers to k determined in pink ghosts made from HbA cells. Full details are given in Section 2.

3.2. Volume regulatory inorganic transport systems

Co- and counter-transporters handling inorganic ions have received considerable attention as volume regulatory effectors [12,13]. Transporters with a net inwardly directed electrochemical gradient (or net chemical gradient only in the case of electroneutral transporters), or which mediate net influx of solute when coupled to anion exchange may be involved in regulatory volume increase (RVI). These systems often involve Na^+, for example NKCC and Na^+/H^+ exchange. Net influx of solutes, with water following osmotically, enables cells to swell after shrinking. NKCC, however, is often poised with no net chemical gradient, especially in human red cells, and so is unable to alter volume. By contrast, transporters with a net outward gradient (like KCC in most red cells) carry out regulatory volume decrease. Previous reviews have emphasised the observation

Table 3
Summary of O_2 sensitivity of membrane transporters in red cells from sickle cell anaemia patients (HbS cells)

	Flux in air	Flux in N_2	Air/N_2	N_2/air	O_2 dependence
GLUT-1	29.54 ± 6.33	25.91 ± 4.75	1.1 ± 0.1	0.9 ± 0.0	0
NKCC	− 0.12 ± 0.18	0.90 ± 0.24	0	∞	−
KCC	3.66 ± 0.08	2.38 ± 0.08	1.6 ± 0.0	0.6 ± 0.0	+
Na^+ pump	0.80 ± 0.36	0.96 ± 0.18	0.8 ± 0.2	1.2 ± 0.3	0
AE1 cell	0.47 ± 0.11	0.40 ± 0.06	1.1 ± 0.1	0.9 ± 0.1	0

Flux via NKCC in air is taken as 0, otherwise see Table 2 legend for details.

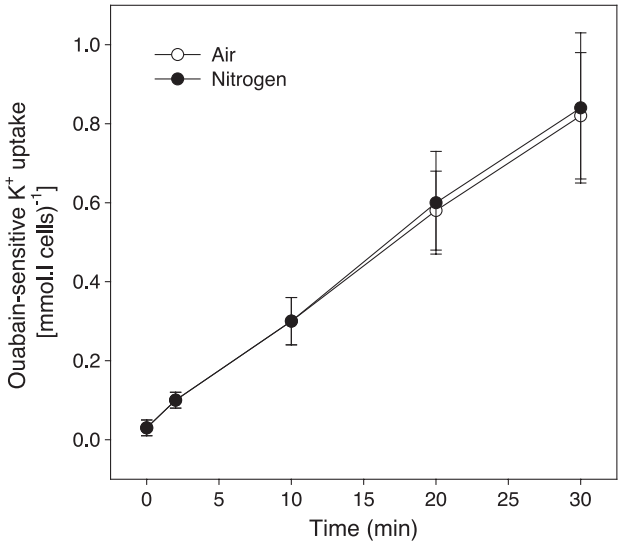

Fig. 1. O_2 sensitivity of Na^+/K^+ pump activity in normal human red cells (HbA cells). Na^+/K^+ pump activity, in mmol (l cells h)$^{-1}$, was determined at 37 °C as the ouabain (100 μM)-sensitive K^+ influx in fully oxygenated or fully deoxygenated HbA cells. Symbols represent means ± S.E.M. ($n = 3$).

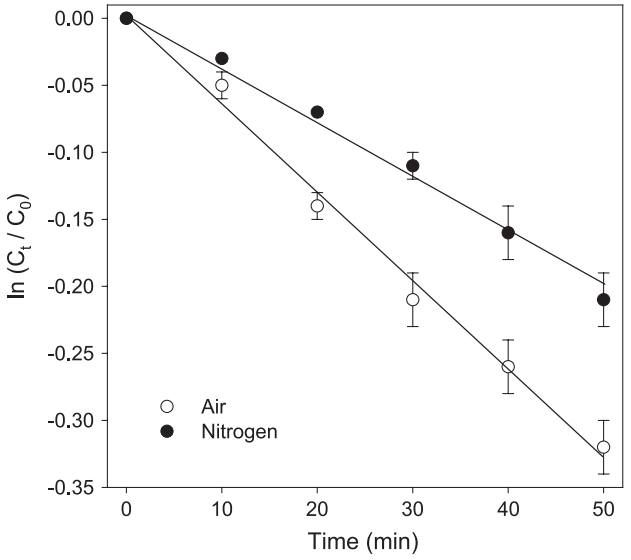

Fig. 3. O_2 sensitivity of the anion exchanger (AE1) in normal human red cells (HbA cells). AE1 activity was determined at room temperature as the rate constant (k) for SO_4^- efflux from fully oxygenated or fully deoxygenated HbA cells. Efflux follows the equation: $(C_\infty - C_t) = (C_\infty - C_0)e^{-kt}$, where C_∞ = total initial intracellular counts radioactivity in cells at time 0, C_t = radioactivity in supernatant at time t, C_0 = sample at time 0, k = rate constant and t = time. The slope of $\ln[(C_\infty - C_t)/(C_\infty - C_0)]$ vs. t gives $-k$; see Section 2. Symbols represent means ± S.E.M. ($n = 7$).

that RVD systems, like KCC, tend to be stimulated at higher O_2 tensions, those mediating RVI by low O_2 tensions [7]. The present results correspond to this general pattern, although it is apparent that the degree of O_2 sensitivity varies across species (cf. KCC in chicken and HbA cells). The apparent reciprocity of RVD and RVI transporters to protein (de)phosphorylation led to the hypothesis that common regulatory enzymes are stimulated (inhibited) by oxygenation (deoxygenation) and thereby apply reciprocal control to these two sets of transporters preventing both

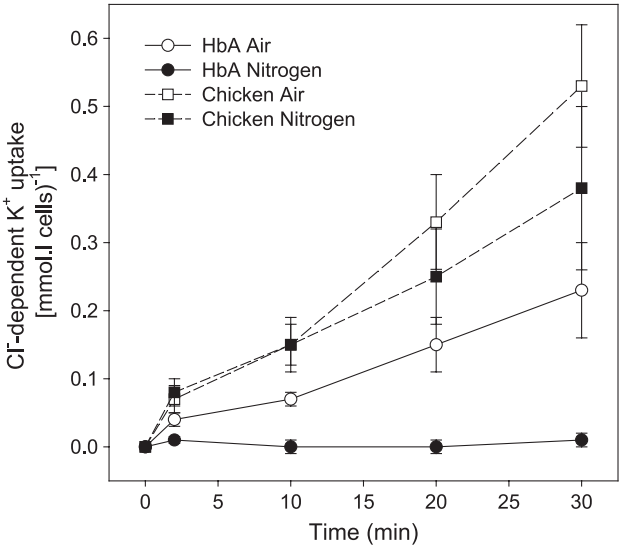

Fig. 2. O_2 sensitivity of $K^+ - Cl^-$ cotransport (KCC) in normal human red cells (HbA cells) and chicken red cells. KCC activity, in mmol (l cells h)$^{-1}$, was determined at 37 °C as the Cl-dependent, bumetanide-insensitive K^+ influx in fully oxygenated or fully deoxygenated HbA cells or chicken red cells. Symbols represent means ± S.E.M. ($n = 3$).

being active simultaneously (which would cause dissipation of ion gradients, with the ensuing metabolic consequence, but without affecting cell volume [13]). Except for O_2 carriage in certain teleosts [14,15], the physiological relevance of this responsiveness to O_2 is not always clear. Inhibition of KCC in more hypoxic regions of the circulation, like active muscle beds, however, may protect against its inappropriate stimulation by low pH. Similarly, O_2 sensitivity of KCC and NKCC may be useful in buffering against large changes in plasma $[K^+]$ [6,16,17].

3.3. Na^+/K^+ pump

In contrast to RVD/RVI systems, the Na^+/K^+ pump shows no difference in activity in response to full oxygenation or deoxygenation. This transporter has a central role for long term volume regulation in most vertebrate cells [18]. It is also used to maintain Na^+ and K^+ concentration gradients, thus enabling the function of excitable cells, and supporting the activity of various secondary active transport systems involving these ions. Further, as explained above, some of these secondary active transporters are stimulated by oxygenation and some by deoxygenation [7]. It is perhaps not surprising therefore that the Na^+/K^+ pump maintains its activity regardless of O_2 tension, responding mainly to altered substrate levels. It is interesting that chicken red cells, which are nucleated and contain mitochondria, and may be expected to lower adenosine triphosphate (ATP) production on deoxygenation, also lack an O_2-

sensitive Na^+/K^+ pump activity, as also observed in trout [19]. This lends support to the hypothesis of privileged pools of ATP [20], perhaps membrane-bound, for regulation of this, and other, transporters.

3.4. Anion exchanger

Like the Na^+/K^+ pump, it is not immediately apparent why AE should show O_2 sensitivity. Thus, AE is required mainly for CO_2 carriage, mediating the exchange of intracellular HCO_3^- for extracellular Cl^- in deoxygenating red cells in peripheral tissues, and vice versa in oxygenating red cells in the lungs [1]. Teleogically, one might expect that both of these processes would require a similar activity of AE. Lack of O_2 sensitivity of Cl^- permeability has been reported previously in red cells from several species of lower vertebrates and also in human [21,22]—which has been ascribed to a flexible link between the domains of band 3 responsible for haemoglobin binding and for anion transport. Table 2 and Fig. 3, however, clearly show that AE activity, as measured by sulphate transport, is greater in intact oxygenated HbA cells (by about 1.5-fold). Similar findings have also been observed [23], although the differences in their report were larger. The difference with previous work on human red cells may reflect the fact that not all Cl^- permeability will be mediated via AE, thus obscuring any effects of oxygenation. It is possible that, when red cells are becoming oxygenated in the lungs, their transit time in the capillaries is faster then in peripheral tissues, making this O_2 sensitivity useful. Interestingly, the O_2 dependence of AE activity was very much reduced in HbA pink ghosts, and also HbS cells, implying a possible role for Hb. We have reviewed elsewhere the evidence for the participation of oxy–deoxy transitions of Hb, acting via a membrane target, possibly the cytoplasmic tail of AE and also involving a number of glycolytic enzymes [7]. O_2-dependent AE activity may contribute to the Haldane effect, hitherto ascribed to the altered buffering power of deoxyHb (being a weaker acid than oxyHb) and formation of carbamate preferentially with deoxyHb. In addition, the effect of O_2 on the activity of AE would allow faster removal of HCO_3^- and hence CO_2 to alveoli. We are currently studying this phenomenon further.

3.5. Organic transporters: GLUT

The final transporter investigated in this paper is the glucose transporter, GLUT. Previous reports in the literature suggest that this system is very O_2-sensitive, being stimulated some five-fold by deoxygenation in avian red cells [24]. Here, we found modest stimulation of GLUT in deoxygenated chicken red cells, but no effect of O_2 tension in human red cells, again illustrating species variations. Only a limited number of other organic transporters have been studied in the context of O_2 sensitivity. Thus, the amino acid transporters ASC and gly were stimulated by oxygenation in human red cells [25], whilst y^+ and y^+L were unaffected [26]. The different transporters showed different kinetic responses, however, with subtle changes in V_{max} and/or K_m. It may be that a more thorough study of GLUT will reveal similar effects. In the case of gly and ASC, it has been proposed that a greater supply of their substrate amino acids is beneficial in oxygenated conditions, as they form substrates for reduced glutathione, the major red cell antioxidant [27]. In the case of glucose, glycolysis is increased in deoxygenation [28], even in mammalian red cells, and hence stimulation of its transporter may be advantageous.

3.6. O_2 sensitivity in red cells from sickle cell patients

HbS cells show abnormalities in the O_2 dependence of KCC, which may be relevant to the pathophysiology of sickle cell disease [9]. In these cells, KCC activity, instead of becoming inactive on deoxygenation, goes through a nadir at about 40 mm Hg, but then re-activates at lower O_2 tensions [8,29]. Thus, KCC activity in fully deoxygenated HbS cells is not dissimilar in magnitude to that in fully oxygenated ones. We confirm those findings here. In addition, we investigated the four other transporters to determine whether their response to O_2 was different in HbA and HbS cells. No qualitative differences were apparent for GLUT-1, NKCC and Na^+/K^+ pump. The higher O_2 sensitivity of NKCC in sickle cells is interesting. Whilst it may be useful to activate an RVI process to maintain cell hydration in the face of deoxygenation-induced solute losses through Gardos channel and KCC, the small net chemical gradient and high Na^+ content of sickle cells will mediate against this. In the case of AE1, however, the O_2 dependence of activity observed in HbA cells was lost in HbS cells. There are many differences in the phosphorylation pattern of membrane proteins between HbA and HbS cells, and these are also affected by O_2 tension [30–32]. If AE1 is indeed a target involved in oxy–deoxy transitions of Hb, then this may explain its loss of O_2 sensitivity in HbS cells.

4. Conclusion

The present findings emphasise the extent to which the response to O_2 is both specific and selective, across species and different transporters. The sensitivity of red cell membrane transporters to O_2 implies a direct link between the O_2 sensor and the transport effector proteins. The concept of a complex cytoskeletal network, also seen in the context of regulation of epithelial transporters (such as CFTR and ENaC, e.g. Ref. [33]), and which acts to coordinate the activity of different transporters, would seem to apply also to red cells. Identifying the mechanism underlying the response to O_2 will be important for our understanding of its physiological relevance, how it is perturbed in disease conditions and how therapeutic intervention may correct abnormalities.

Acknowledgements

We thank Action Research and The Wellcome Trust for financial support.

References

[1] M. Nikinmaa, Vertebrate red blood cells, in: S.D. Bradshaw, W. Heller, H.C. Heller, S. Ishii, H. Langer, G. Neuweiler, D.J. Randall (Eds.), Zoophysiology, vol. 28, Springer, Berlin Heidelberg, 1990, pp. 45–46.
[2] S.L. Orskov, The potassium absorption by pigeon blood cells, Acta Physiol. Scand. 31 (1954) 221–229.
[3] D.C. Tosteson, J.S. Robertson, Potassium transport in duck red cells, J. Cell. Comp. Physiol. 47 (1956) 147–166.
[4] R. Motais, F. Garcia-Romeu, F. Borgese, The control of Na/H exchange by molecular oxygen in trout erythrocytes, J. Gen. Physiol. 90 (1987) 197–207.
[5] F. Borgese, R. Motais, F. Garcia-Romeu, Regulation of Cl-dependent K transport by oxy–deoxyhemoglobin transitions in trout red cells, Biochim. Biophys. Acta 1066 (1991) 252–256.
[6] O.B. Nielsen, G. Lykkeboe, A.R. Cossins, Oxygenation-activated K^+ fluxes in trout red blood cells, Am. J. Physiol. 263 (1992) C1057–C1064.
[7] J.S. Gibson, A.R. Cossins, J.C. Ellory, Oxygen-sensitive membrane transporters in vertebrate red cells, J. Exp. Biol. 203 (2000) 1395–1407.
[8] J.S. Gibson, P.F. Speake, J.C. Ellory, Differential oxygen sensitivity of the K^+–Cl^- cotransporter in normal and sickle human red blood cells, J. Physiol. 511 (1998) 225–234.
[9] J.S. Gibson, J.C. Ellory, Membrane transport in sickle cell disease, Blood Cells Mol. Diseases 28 (2002) 1–12.
[10] P.B. Dunham, J.C. Ellory, Passive potassium transport in low potassium sheep red cells: dependence upon cell volume and chloride, J. Physiol. 318 (1981) 511–530.
[11] S. Lepke, H. Fasold, M. Pring, H. Passow, A study of the relationship between inhibition of anion exchange and binding to the red blood cell membrane of 4,4′ -diisothiocyano stilbene-2,2′ -disulfonic acid (DIDS) and its dihydro derivative (H_2DIDS), J. Membr. Biol. 29 (1976) 147–177.
[12] E.K. Hoffmann, L.O. Simonsen, Membrane mechanisms in volume and pH regulation in vertebrate cells, Physiol. Rev. 69 (1989) 315–382.
[13] A.R. Cossins, J.S. Gibson, Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells, J. Exp. Biol. 200 (1997) 343–352.
[14] M. Nikinmaa, Membrane transport and control of hemoglobin-oxygen affinity in nucleated erythrocytes, Physiol. Rev. 72 (1992) 301–321.
[15] M. Berenbrink, S. Volkel, N. Heisler, M. Nikinmaa, O_2-dependent K^+ fluxes in trout red blood cells: the nature of O_2 sensing revealed by the O_2 affinity, cooperativity and pH dependence of transport, J. Physiol. 526 (2000) 69–80.
[16] P.F. Speake, C.A. Roberts, J.S. Gibson, Effect of changes in respiratory blood parameters on equine red blood cell K–Cl cotransporter, Am. J. Physiol. 273 (1997) C1811–C1818.
[17] M.C. Muzyamba, A.R. Cossins, J.S. Gibson, Regulation of Na^+–K^+–$2Cl^-$ cotransport in turkey red cells: the role of oxygen tension and protein phosphorylation, J. Physiol. 517 (1999) 421–429.
[18] E.K. Hoffmann, P.B. Dunham, Membrane mechanisms and intracellular signalling in cell volume regulation, Int. Rev. Cytol. 161 (1995) 173–262.
[19] J. Pesquero, T. Roig, J. Sanchez, J. Bermudez, Na(+)–K(+) pump and metabolic activities of trout erythrocytes during anoxia, Am. J. Physiol. 277 (1999) C29–C34.
[20] J.F. Hoffman, ATP compartmentalization in human erythrocytes, Curr. Opin. Hematol. 4 (1997) 112–115.
[21] F.B. Jensen, J. Brahm, Kinetics of chloride transport across fish red blood cell membranes, J. Exp. Biol. 198 (1995) 2237–2244.
[22] F.B. Jensen, T. Wang, D.R. Jones, J. Brahm, Carbon dioxide transport in alligator blood and its erythrocyte permeability to anions and water, Am. J. Physiol. 274 (1998) R661–R671.
[23] A. Galtieri, E. Tellone, L. Romano, F. Misiti, E. Bellocco, S. Ficarra, A. Russo, D. DiRosa, M. Castagnola, B. Giardina, I. Messana, Band-3 protein function in human erythrocytes: effect of oxygenation–deoxygenation, Biochim. Biophys. Acta 1564 (2002) 214–218.
[24] R.E. Wood, H.E. Morgan, Regulation of sugar transport in avain erythrocytes, J. Biol. Chem. 244 (1969) 1451–1460.
[25] K. Kiessling, N. Roberts, J.S. Gibson, J.C. Ellory, A comparison in normal individuals and sickle cell patients of reduced glutathione precursors and their transport between plasma and red cells, Hematol. J. 1 (2000) 243–249.
[26] K. Kiessling, The importance of amino acid transport for human red blood cells, D.Phil. thesis (1997) University of Oxford, UK.
[27] H. Sies, Oxidative stress: oxidants and antioxidants, Exp. Physiol. 82 (1997) 291–295.
[28] I. Messana, M. Orlando, L. Cassiano, L. Pennacchietti, C. Zuppi, M. Castagnola, B. Giardina, Human erythrocyte metabolism is modulated by the O_2-linked transition of haemoglobin, FEBS Lett. 390 (1996) 25–28.
[29] J.S. Gibson, A. Khan, P.F. Speake, J.C. Ellory, O_2 dependence of K^+ transport in sickle cells: the effects of different cell populations and the substituted benzaldehyde, 12C79, FASEB J. 15 (2001) 823–832.
[30] H. Fathallah, E. Coezy, R.-S. de Neef, M.-D. Hardy-Dessources, F. Giraud, Inhibition of deoxygenation-induced membrane protein dephosphorylation and cell dehydration by phorbol esters and okadaic acid in sickle cells, Blood 86 (1995) 1999–2007.
[31] P. Merciris, M.-D. Hardy-Dessources, M. Sauvage, F. Giraud, Involvement of deoxygenation-induced increase in tyrosine kinase activity in sickle cell dehydration, Eur. J. Phys. 436 (1998) 315–322.
[32] P. Merciris, M.-D. Hardy-Dessources, F. Giraud, Deoxygenation of sickle cells stimulates Syk tyrosine kinase and inhibits a membrane tyrosine phosphatase, Blood 98 (2001) 3121–3127.
[33] D. Pearce, SGK1 regulation of epithelial sodium transport, Cell. Physiol. Biochem. 13 (2003) 13–20.