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Anion movements across lamprey (*Lampetra fluviatilis*) red cell membrane

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Chloride and bicarbonate movements across lamprey red cell membrane were investigated. The halftime for equilibration of radioactive chloride across the red cell membrane was 2.46 h, and apparent permeability for chloride-36 was approximately 10^{-9} cm • s^{-1}, a value similar to that observed for lipid bilayers. Chloride movements were not affected by the anion exchange inhibitor, 4,4′-diisothiocyano-stilbene-2,2′-disulfonic acid (DIDS). Furthermore, intracellular buffering is effectively isolated from the extracellular compartment, as shown by the fact that practically no pH recovery occurred in the unbuffered extracellular medium after either acidification or alkalinization. These observations show that lamprey red cell membrane is quite impermeable to bicarbonate and other acid/base equivalents.

Lamprey erythrocytes regulate their intracellular pH by a sodium-dependent process [1,2]. This is possible if either the sodium-dependent acid extrusion is extremely effective or if the anion exchange pathway, equilibrating acid equivalents across the red cell membrane and characteristic for other red cells studied, is lacking in lamprey red cells, as suggested by Ohnishi and Asai [3]. In the present study we have investigated the movements of chloride and bicarbonate across the red cell membrane of lamprey to characterize further the factors affecting the red cell pH in lamprey.

River lampreys (*Lampetra fluviatilis*; $n = 100$; 20–100 g) were caught during their spawning run and maintained in laboratory conditions (T 6–10 °C, air-saturated water, pH 7.2–7.5) for a minimum of two weeks before experimentation. Blood samples were taken by venipuncture from anaesthetized (2 g MS222/l water; 2 min) animals. Red cells and plasma were separated by centrifugation, and red cells washed twice with the buffer used in the experiments (30 mM Hepes, 110 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 g/l glucose, 1 g/l sodium pyruvate and 1 g/l sodium butyrate). All the experiments were carried out at 20 °C.

Chloride movements across the red cell membrane were determined using radioactive chloride-36. Red cells were suspended to a haematocrit value of 5 in the buffer used, and 5 μl/ml suspension of radioactive chloride added to the incubation (the activity of the suspension was 9.15 μCi/l). The suspension was shaken continuously, and aliquots taken after 5, 10, 30 and 60 min, and thereafter after 3, 6 and 24 h. The cells and incubation medium were separated by centrifugation, the red cells weighed, and deproteinized with 0.6 M perchloric acid. Both the incubation medium and cells were counted for chloride-36 (LKB Minibeta 1211). The intracellular chloride counts were corrected for extracellular trapped water,

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measured as polyethylene glycol space

$$\text{trapped } H_2O(\%) = \frac{(\text{DPM } ^3\text{H/ml cell pellet})}{(\text{DPM } ^3\text{H/ml supernatant})}$$

The activity of chloride-36 was given as μCi/l red cell water. The red cell water was determined by weighing the cells, drying them to a constant weight and reweighing (see Ref. 4).

The data were fitted to the equation (see for example, Ref. 5)

$$A_t = A_e(1 - e^{-kt})$$

in which A_t = the intracellular activity of chloride-36 at time t, A_e = the intracellular activity of chloride-36 at equilibrium, k = the observed rate constant and t = equilibration time, using a modification of Spain's [6] Curfit program.

The radioactivity appearing in the cells as a function of time is given in Fig. 1. The best fitting

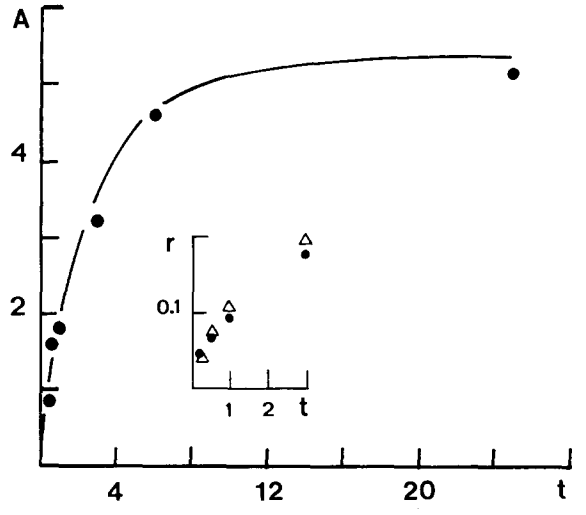

Fig. 1. Equilibration of chloride-36 across lamprey red cell membrane. A = intracellular activity of chloride-36 (in μCi/l red cell water), t = time (in hours). The experimental points (means of 5–10 experiments) were fitted to the equation

$$A_t = A_e(1 - e^{-kt})$$

giving a value of 5.23 for A_e and 0.407 for k. The inset gives the ratio (r) $^{36}Cl^-$ (cell; μCi/l cell water)/$^{36}Cl^-$ (medium; μCi/l water) for control cells (●) and cells treated with 10^{-4} M DIDS (△) as a function of time (t). Values are means of seven experiments. For further details see text.

curve for the relation between radioactivity and time is

$$A_t = 5.23(1 - e^{-0.407t})$$

giving the rate constant of 0.407 and halftime of 2.46 h for the equilibration of chloride-36 through the membrane. In rainbow trout, at a slightly lower temperature, 15 °C, the halftime for chloride equilibration is only 0.8 seconds [7] – only 1/10 000 of that in lamprey red cells at 20 °C. The initial influx of chloride (into one liter of red cell water), calculated on the basis of 118 mM extracellular chloride concentration is 48 mmol chloride/h.

An estimate of the permeability of lamprey erythrocytes to chloride is derived by multiplying the rate constant with the ratio V/A (the volume of intracellular solution/surface area of the cell; see Ref. 5). Lamprey red cells are approximately half-spherical in shape, with a mean volume of approx. 250 μm^3. From these observations, a very rough estimate of V/A of 10^{-5} cm is obtained, giving a value of 10^{-9} cm · s^{-1} for the permeability of lamprey red cells to chloride. Interestingly, this value is similar to that observed for lipid bilayers at the same temperature [8]. These results show that chloride movements across lamprey red cell membrane are exceedingly slow in comparison to those of other red cells studied, but similar to those in lipid bilayers.

The chloride movements are not affected by treating the cells with DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid), an inhibitor of anion exchange pathway. The cells, suspended to a haematocrit value of 5, were incubated for 30 min in the presence or absence of DIDS before chloride-36 was added to the incubation. Thereafter the appearance of radioactivity in the cells was followed for 3 h as described above, and the ratio chloride-36 (cell)/chloride-36 (medium) calculated for each time point. As seen from Fig. 1 (inset), within this time period the ratio of cellular chloride-36 to chloride-36 in the medium was similar in both DIDS-treated and control cells.

Two additional experiments were carried out to investigate the possible role of bicarbonate movements in affecting intracellular pH. Firstly, red

cells, treated as above, were suspended into a haematocrit of 3 in unbuffered sucrose solution (280 mosM), and changes in the extracellular pH occurring after the addition of red cells followed (see Ref. 9). In the presence of chloride/bicarbonate exchange, intracellular chloride is rapidly exchanged for extracellular bicarbonate, and the steady-state pH of extracellular medium decreases markedly. This was seen using rainbow trout red cells, as also observed by Cossins and Richardson [9], but not in lamprey red cells (not shown), indicating that chloride/bicarbonate exchange does not exist in lamprey red cells. Secondly, base or acid was added into unbuffered red cell suspension at pH equilibrium (the buffer used was the same as above, but without Hepes). If rapid movements of bicarbonate or other acid/base equivalents occurred, intracellular buffering (mainly haemoglobin) would largely 'absorb' the acid or base load, and extracellular pH would rapidly decrease after the initial alkalinization or increase after the initial acidification. Such a situation was observed in trout red cells (Fig. 2a), treated as described by Cossins and Richardson [9], whereas in the experiment with lamprey red cells practically no recovery was observed either after initial alkalinization or acidification (Fig. 2b). This finding indicates that intracellular buffering is effectively isolated from the extracellular compartment, i.e. the movements of acid/base equivalents into the cell are slow.

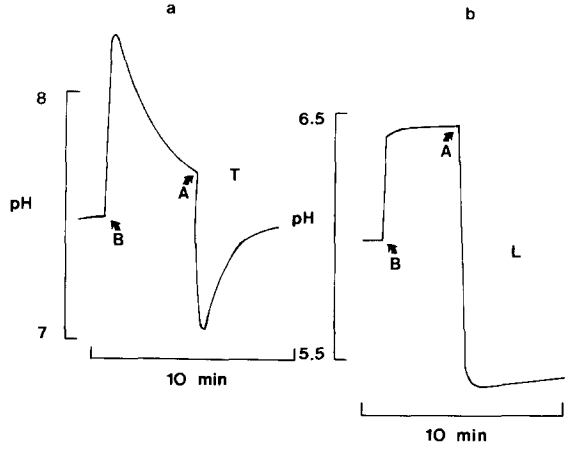

Fig. 2. The effects of base (B: 0.001 M NaOH) and acid (A: 0.001 M HCl) addition on the extracellular pH of trout (a) and lamprey (b) red cells incubated in unbuffered extracellular medium. The figures are representative of six experiments.

The relative impermeability of lamprey erythrocytes to acid/base equivalents thus appears to be one of the factors making the red cell pH, in short term, insensitive to variations in external pH, induced by mineral acid or base [1]. It is likely that in physiological situations the internal acid loads of lamprey red cells are mainly generated by carbon dioxide entering the cells, and dissociating to protons and bicarbonate. We are presently investigating the responses of lamprey red cells to carbon dioxide-induced acidification.

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