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ACUTE AND CHRONIC INFLUENCE OF TEMPERATURE ON RED BLOOD CELL ANION EXCHANGE

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Summary

Unidirectional $^{36}Cl^-$ efflux *via* the red blood cell anion exchanger was measured under Cl^- self-exchange conditions (i.e. no net flow of anions) in rainbow trout *Oncorhynchus mykiss* and red-eared freshwater turtle *Trachemys scripta* to examine the effects of acute temperature changes and acclimation temperature on this process. We also evaluated the possible adaptation of anion exchange to different temperature regimes by including our previously published data on other animals. An acute temperature increase caused a significant increase in the rate constant (*k*) for unidirectional Cl^- efflux in rainbow trout and freshwater turtle. After 3 weeks of temperature acclimation, 5 °C-acclimated rainbow trout showed only marginally higher Cl^- transport rates than 15 °C-acclimated trout when compared at the same temperature. Apparent activation energies for red blood cell Cl^- exchange in trout and turtle were lower than values reported in endothermic animals. The Q_{10} for red blood cell anion exchange was 2.0 in trout and 2.3 in turtle, values close to those for CO_2 excretion, suggesting that, in ectothermic animals, the temperature sensitivity of band-3-mediated anion exchange matches the temperature sensitivity of CO_2 transport (where red blood cell Cl^-/HCO_3^- exchange is a rate-limiting step). In endotherms, such as man and chicken, Q_{10} values for red blood cell anion exchange are considerably higher but are no obstacle to CO_2 transport, because body temperature is normally kept constant at values at which anion exchange rates are high. When compared at constant temperature, red blood cell Cl^- permeability shows large differences among species (trout, carp, eel, cod, turtle, alligator, chicken and man). Cl^- permeabilities are, however, remarkable similar when compared at preferred body temperatures, suggesting an appropriate evolutionary adaptation of red blood cell anion exchange function to the different thermal niches occupied by animals.

Key words: anion exchange, Cl^- transport, erythrocyte, temperature, CO_2 transport, ectotherm, endotherm, *Oncorhynchus mykiss*, *Trachemys scripta*.

Introduction

The presence of an anion exchange protein (known as AE1 or band 3) in the membrane of vertebrate red blood cells (RBCs) endows the RBC membrane with a high permeability to Cl^- and HCO_3^-. This makes the intracellular buffering capacity available for extracellular acid–base loads and is important for CO_2 transport in the blood. Metabolically produced CO_2 diffuses into the RBCs in tissue capillaries and is rapidly hydrated to H_2CO_3 (catalysed by carbonic anhydrase), which dissociates to H^+ and HCO_3^-. The binding of H^+ to haemoglobin and the shift of the HCO_3^- produced to the plasma (in exchange for Cl^- *via* AE1) both drive the equilibrium reaction further towards HCO_3^- formation, elevating the CO_2-carrying capacity of the blood. In capillaries at the respiratory surfaces, plasma HCO_3^- re-enters the RBCs, and the concomitant release of H^+ from haemoglobin leads to the re-formation of CO_2 that subsequently diffuses out across the respiratory epithelium. The Cl^-/HCO_3^- exchange is considered the rate-limiting step, and it must be very fast to reach completion during the short transit time of the blood in the capillaries (e.g. Wieth et al., 1982).

The anion exchange process is strongly temperature-sensitive in human RBCs (Brahm, 1977), with unidirectional anion fluxes *via* AE1 decreasing to very low values at low temperatures. In the light of this, it was interesting to observe that the Cl^- permeability of some fish RBCs at 15 °C was very close to that of human RBCs at 37 °C (Jensen and Brahm, 1995). Similarly, in alligators (*Alligator mississippiensis*), the apparent Cl^- and HCO_3^- permeabilities at 30 °C were close to corresponding values in man at 37 °C (Jensen et al., 1998). This suggests an appropriate adaptation of band-3-mediated anion transport to the different temperature regimes encountered by the animals. The thermal niche of ectothermic animals is variable and complex. Different species may live in different thermal habitats and can experience changes in body

temperature on both a short-term (e.g. diurnal) and a long-term (e.g. seasonal) basis. Against this background, we found it of interest to study both the acute and chronic effects of temperature on RBC anion transport. The major aims of the study were (i) to test whether the acute temperature sensitivity of RBC Cl^- exchange in rainbow trout and freshwater turtle is reduced compared with that of endothermic animals such as man and chicken; (ii) to investigate whether temperature acclimation within a given species (rainbow trout) has an influence on RBC anion transport; (iii) to gain further insight into the possible adaptation of band 3 function to different thermal niches (by examining ectotherms with different thermal preferences); and (iv) to evaluate the temperature sensitivity of RBC anion exchange in ectotherms in relation to its major role in blood CO_2 transport.

Materials and methods

Experimental animals and temperature acclimation

Trout

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)], weighing 90.1±24.1 g (mean ± S.D., *N*=130), were obtained from a fish farm in Jutland, Denmark. The fish were acclimated at a water temperature of 11 °C to Odense tapwater in 400 l tanks with a continuous inflow of fresh tapwater. After 1–2 weeks, the fish were randomly selected and transferred to aquaria containing 60 l of water thermostatted at either 5 °C or 15 °C. Each aquarium contained approximately 15 fish, and 40 l of the water was renewed daily. The fish were temperature-acclimated for 3 weeks at 5 °C or 15 °C, and the water was kept normoxic (P_{O_2}>135 mmHg=18.0 kPa) by bubbling with air.

Turtles

Specimens of the red-eared freshwater turtle *Trachemys scripta* Gray, weighing 400–700 g (*N*=4), were held in a 400 l tank with water for submergence and platforms for terrestrial contact. A heat lamp provided a basking site for thermoregulation, allowing body temperature to be maintained at approximately 20–35 °C. The preferred body temperature of *Trachemys scripta* (formerly named *Pseudemys scripta*) is near 30 °C (Hammond et al., 1988).

Experimental protocol

Blood was drawn from the caudal vessels of rainbow trout into heparinised syringes and pooled in 10 ml test tubes. The fish were killed by a sharp blow to the head. In turtles, blood was obtained directly from the carotid artery of anaesthetised (100 mg kg^{-1} intraperitonally applied Nembumal) animals. Some 25–40 ml of blood was obtained from each turtle, whereupon the animal was killed by infusion of saturated KCl.

The blood was centrifuged and the plasma removed. The osmolality of the plasma was measured using a cryoscopic osmometer (Gonotec Osmomat 030), and a saline with the same osmolality as the plasma was prepared by mixing appropriate amounts of a high-osmolality saline with low-osmolality saline. The composition of the high-osmolality saline was (in mmol l^{-1}): NaCl, 200; $NaHCO_3$, 6; KH_2PO_4, 2.9; $CaCl_2$, 2; $MgSO_4$, 1; glucose, 3.9; Hepes buffer, 10. The low-osmolality saline had the same composition except that [NaCl] was 100 mmol l^{-1}. Red blood cells (RBCs) were washed three times in iso-osmotic saline and suspended in the saline at a haematocrit of 50 %. These RBC suspensions were equilibrated for 45 min in an Eschweiler (Kiel, Germany) tonometer with humidified air (oxygenating the cells). The tonometer was thermostatted at 5 °C, 15 °C or 22 °C in experiments with trout RBCs and at 15 °C, 25 °C and 35 °C in experiments with turtle RBCs. Some 20 min prior to the end of the equilibration period, the isotope $^{36}Cl^-$ (as NaCl) was added (final radioactivity 15 kBq ml^{-1}). Following equilibration, pH was measured with the capillary pH electrode of a Radiometer (Copenhagen, Denmark) BMS 3 electrode set-up, which was thermostatted at the equilibration temperature. The RBC suspension was subsequently centrifuged for 10 min at 3850 ***g*** to obtain packed RBCs for $^{36}Cl^-$ efflux experiments.

The rate of tracer efflux was determined at the different experimental temperatures by the continuous flow tube method, which has a time resolution of milliseconds (Brahm, 1977; Brahm, 1989). This is essential because unidirectional Cl^- fluxes *via* the RBC anion exchanger greatly exceed in quantity and are much faster than the Cl^- flux rate *via* other transporters. In short, 0.5 ml of packed and radioactively labelled RBCs was continuously mixed with 270 ml of isotope-free saline in a mixing chamber. The saline had the same temperature as that prevailing during RBC equilibration. The dilute suspension flowed at a known velocity through a tube in which cell-free filtrates were collected at predetermined distances. The filtrates contained increasing amounts of isotope with increasing distance from the mixing chamber. The distances were converted to times using the known flow rate. Radioactivity was measured by β-scintillation spectrometry (Tricarb, Packard Instruments), and the rate of $^{36}Cl^-$ efflux was determined by linear regression of semilogarithmic plots of extracellular radioactivity *versus* time (see Brahm, 1977; Brahm, 1989; Jensen and Brahm, 1995).

The experiments were conducted under Cl^- self-exchange (equilibrium exchange) conditions, i.e. the unidirectional efflux of Cl^- (both non-radioactive Cl^- and the negligible amount of radioactive Cl^-) is equal to the unidirectional influx of Cl^-. Under physiological conditions at rest, the anion exchanger performs approximately 83 % self-exchange of Cl^-, 15 % self-exchange of HCO_3^- and only approximately 2 % hetero-exchange of Cl^- for HCO_3^- (Wieth et al., 1982). The transport capacity occupied by Cl^- and HCO_3^-, respectively, is similar to the ratio between the two ion concentrations, because the relative affinities of the two anions for the transporter are similar (Gasbjerg and Brahm, 1991; Gasbjerg et al., 1996; Knauf et al., 1996).

Results

The Cl^- efflux experiments on rainbow trout RBCs were conducted at pH values (means ± S.E.M.) of 7.70±0.02 at 5 °C,

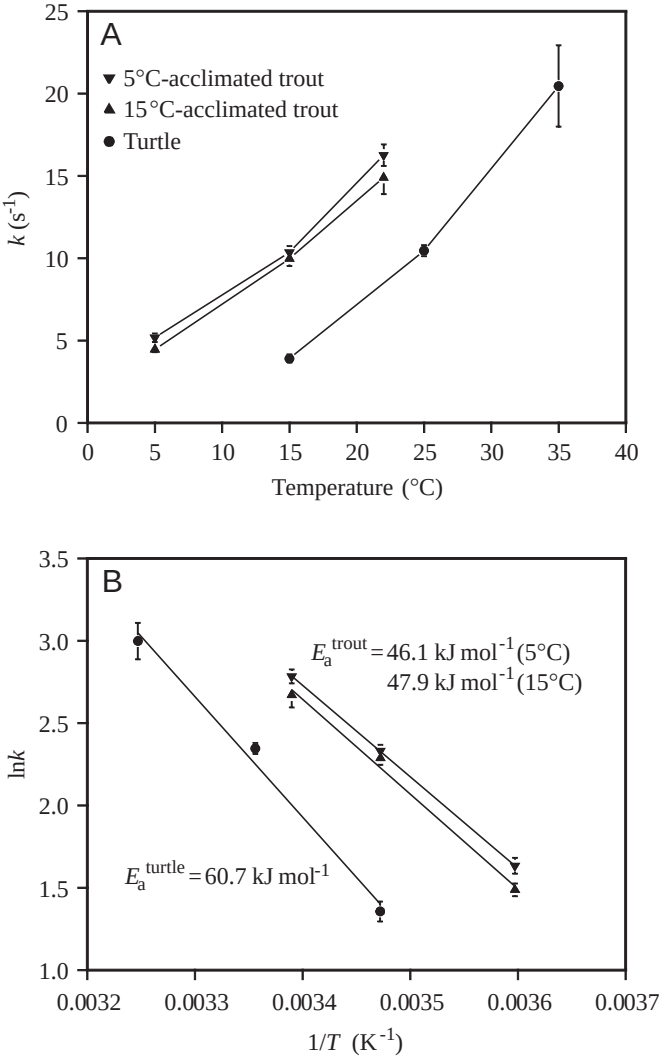

Fig. 1. (A) Temperature dependency of rate coefficients (*k*) for unidirectional Cl^- efflux from rainbow trout (*Oncorhynchus mykiss*) and turtle (*Trachemys scripta*) red blood cells. Cl^- efflux was measured under self-exchange conditions (see text for further details). (B) Arrhenius plots (ln*k* *versus* 1/*T*, where *T* is absolute temperature) of the data, depicting the calculated activation energies (E_a=–slope×***R***, where ***R*** is the gas constant). Values are means ± S.E.M. (the number of replicates at each point was 7–15 for trout and four for turtle). Extracellular pH values were 7.6–7.7 for both species.

7.66±0.03 at 15 °C and 7.58±0.02 at 22 °C. The minor differences in pH, resulting from the different measurement temperatures, do not influence Cl^- transport kinetics (Jensen and Brahm, 1995). The rate constant (*k*) for unidirectional $^{36}Cl^-$ efflux from the RBCs increased as a function of temperature for both 5 °C- and 15 °C-acclimated trout (Fig. 1A). Two-factor analysis of variance on *k* values revealed that the influence of measurement temperature was significant (P<0.001), whereas the influence of acclimation temperature was not (P=0.099). Following a logarithmic transformation to ln*k*, a significant influence of acclimation temperature appeared (P=0.02). The difference between the two temperature acclimation groups was, however, small (see Fig. 1). The Arrhenius plot showed a linear relationship between ln*k* and the inverse of absolute temperature from which apparent activation energies (E_a) of 46.1 kJ mol^{-1} for 5 °C-acclimated trout and 47.9 kJ mol^{-1} for 15 °C-acclimated trout were calculated (Fig. 1B).

In turtle RBCs, *k* for unidirectional Cl^- efflux also increased significantly with temperature but, at a given temperature (e.g. 15 °C), *k* values were significantly lower (P<0.01) than in rainbow trout (Fig. 1A). The E_a for Cl^- transport in turtle RBCs was 60.7 kJ mol^{-1} (Fig. 1B).

The size of RBCs varies among species. To enable better comparison among species, the rate coefficients *k* (s^{-1}) were therefore converted into apparent permeability coefficients P_{Cl} (μm s^{-1}) by taking into account the ratio of cell water volume (V_w) to membrane surface area (A_m):

$$P_{Cl} = k(V_w/A_m)\,. \quad (1)$$

The ratio V_w/A_m was assumed to be constant in the temperature range considered, and a V_w/A_m value of 0.555 μm was adopted for rainbow trout RBCs from the data of Jensen and Brahm (1995). In turtle RBCs, a V_w/A_m ratio of 0.631 μm was used, resulting from a mean cellular volume of 327 μm^3 (Yamaguchi et al., 1989), a measured water fraction of 0.673 and a value of A_m of 348.7 μm^2 (calculated as in Jensen and Brahm, 1995). An Arrhenius plot of P_{Cl} in trout and turtle is shown in Fig. 2, together with permeability coefficients for Cl^- transport in human RBCs. The latter were calculated from published half-times ($t_{1/2}$=ln2/*k*) and a V_w/A_m value for human RBCs of 0.43 μm (Brahm, 1977). At a given constant temperature (e.g. 15 °C), the Cl^- permeability coefficients followed the sequence: $P_{Cl}^{trout} > P_{Cl}^{turtle} > P_{Cl}^{man}$.

If the intracellular Cl^- concentration varies little with temperature, which seems a reasonable assumption in rainbow trout (Houston and Koss, 1984), then the permeability coefficients will be proportional to actual fluxes. The Q_{10} for Cl^- transport *via* the anion exchanger was accordingly estimated from the equation:

$$Q_{10} = (P_2/P_1)^{10/(T_2-T_1)}\,, \quad (2)$$

where P_2 and P_1 are the permeability coefficients at respective temperatures T_2 and T_1. The data on rainbow trout RBCs give a Q_{10} of 2.0 in the temperature interval 5–22 °C, whereas the average Q_{10} in turtle RBCs is 2.3 (between 15 and 35 °C). In human RBCs, Q_{10} is 7.2 between 0 and 15 °C and 3.4 between 15 and 38 °C, with an average Q_{10} value of 4.7 for the whole temperature interval (Fig. 2).

Discussion

An acute temperature increase had a clear stimulating effect on Cl^- transport *via* the RBC anion exchanger in both rainbow trout and freshwater turtles. In contrast, 3 weeks of temperature acclimation in rainbow trout had only a small influence, characterised by a modest increase in *k* and apparent Cl^- permeability at a given temperature upon cold acclimation

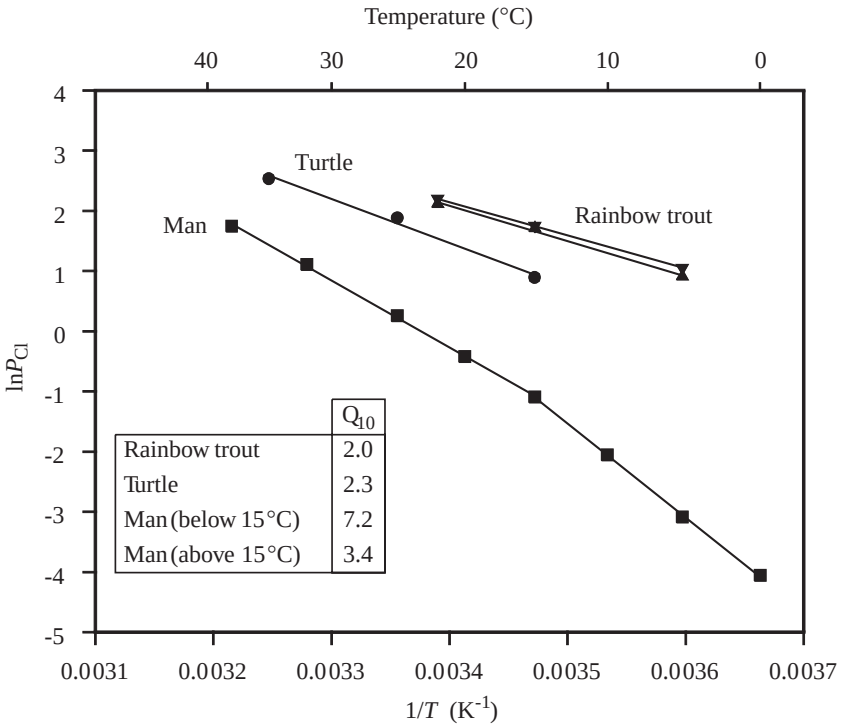

Fig. 2. Temperature dependency of the apparent Cl^- permeability (P_{Cl}, $\mu m\,s^{-1}$), shown as Arrhenius plots ($\ln P_{Cl}$ *versus* $1/T$, where T is absolute temperature), in rainbow trout, turtle and human red blood cells. Human data are based on data from Brahm (Brahm, 1977). The inset shows Q_{10} values for Cl^- exchange calculated from the P_{Cl} values (see text for further details).

(Figs 1, 2). The limited effect of acclimation temperature on band-3-mediated Cl^- transport compares with an insignificant effect of thermal acclimation upon Cl^--dependent K^+ flux (K^+/Cl^- cotransport) in trout RBCs (Raynard and Cossins, 1991), but contrasts with a clear effect of temperature acclimation on Na^+/K^+ pump activity. Cold-acclimated trout have RBCs with a higher rate of ouabain-sensitive K^+ influx than that of warm-acclimated trout RBCs because of an increased turnover number of individual Na^+/K^+ pumps rather than a changed pump number (Raynard and Cossins, 1991).

The E_a of 46–48 kJ mol^{-1} for Cl^- transport in trout RBCs (Fig. 1B) is slightly lower than the E_a of 62.8 kJ mol^{-1} reported for SO_4^{2-} equilibrium exchange (Romano and Passow, 1984), and it compares with the E_a of 55.2 kJ mol^{-1} for HCO_3^-/Cl^- exchange in dogfish (*Mustelus canis*) RBCs (Obaid et al., 1979). The E_a in turtle RBCs (60.7 kJ mol^{-1}, Fig. 1B) is of similar magnitude to that seen in fish RBCs. These values are, however, lower than E_a values of 126 kJ mol^{-1} (below 15 °C) and 84 kJ mol^{-1} (above 15 °C) in human RBCs (Brahm, 1977) and of 138 kJ mol^{-1} (below 20 °C) and 96 kJ mol^{-1} (above 20 °C) in chicken RBCs (Brahm and Wieth, 1977).

Anion exchange is a rate-limiting step in CO_2 excretion in both man and lower vertebrates (e.g. Wieth et al., 1982; Perry, 1986; Jensen and Brahm, 1995). The temperature sensitivity of the anion exchange process is therefore best evaluated in a physiological context by calculating Q_{10} values and comparing them with Q_{10} values for blood CO_2 transport and CO_2 excretion. Q_{10} for oxygen uptake and carbon dioxide excretion typically assumes values of 2–3. A Q_{10} value for band-3-mediated anion exchange higher than this would be inappropriate in ectothermic vertebrates, because cooling would lead to an excessive decrease in HCO_3^-/Cl^- exchange that would be disadvantageous. This is because anion exchange would not approach equilibrium during the transit time of the blood in the capillaries (where gas exchange occurs), and the continuation of the fluxes in the closed (no gas exchange) system of arteries and veins would cause major changes in P_{CO_2} and pH. Indeed, the actual Q_{10} of 2 for RBC Cl^- exchange in rainbow trout seems to match the Q_{10} of approximately 2 for gas transport in this species (Fry, 1971; Evans, 1990). This is also the case for turtle, in which the Q_{10} of 2.3 for RBC anion exchange is similar to the Q_{10} of 2.6 for CO_2 excretion (calculated from the data of Kinney et al., 1977). Thus, the degree of rate-limitation offered by RBC anion exchange in CO_2 transport does not change appreciably as temperature changes within the temperature intervals considered here.

In humans, Q_{10} for RBC Cl^- transport is higher than Q_{10} for gas transport (Fig. 2), but this presents no problem, because body temperature does not normally fall below 37 °C and, at this temperature, the rate of anion exchange is high. This also applies to the chicken, in which the temperature sensitivity of RBC anion exchange is high and similar to that in humans (Brahm and Wieth, 1977; see also Fig. 3). Thus, a picture emerges of a close match between Q_{10} values for gas transport and RBC anion exchange in ectothermic animals, in which a change in body temperature is a factor that has to be dealt with, while this is not the case in endothermic mammals and birds that are able to control their body temperature within narrow limits.

From an evolutionary point of view, our data suggest that a Q_{10} of approximately 2 for AE1-mediated anion transport is an ‘ancient’ feature that adapts anion exchange function in ectothermic animals to the variable CO_2 transport requirements

when body temperature, and thus metabolic rate, changes. Apparently, this trait was lost during the evolution of endothermy. There has probably not been selection pressure for higher Q_{10} values in endothermic animals, but the occasional development of high Q_{10} values may not have been selected against, because body temperature does not vary to any significant extent. With this in mind, it would be interesting to evaluate the temperature sensitivity of RBC anion exchange in hibernating mammals (hedgehogs, hamsters, etc.) that allow their body temperature and metabolic rate to drop to low values during winter. These animals may have retained a Q_{10} for RBC anion exchange that resembles the Q_{10} for metabolic rate.

When the Q_{10} for AE1-mediated anion transport matches the Q_{10} for CO_2 transport, there would be a limited need for further adjustments of anion exchange function during temperature acclimation. Indeed, the small influence of acclimation temperature on Cl^- transport variables observed in trout supports this idea. It should be noted, however, that compensatory changes in metabolic rate may occur during acclimation (e.g. Evans, 1990), and the small effect of temperature acclimation actually seen may reflect a fine adjustment of anion exchange function to such a change.

A Q_{10} for anion exchange of 2 between 5 °C and 22 °C in rainbow trout RBCs is not a matter of course. Various ion transport pathways in trout erythrocytes show quite different Q_{10} values. Adrenergic H^+ extrusion *via* the Na^+/H^+ exchanger has a Q_{10} above 5 (Cossins and Kilbey, 1990) and the Q_{10} for furosemide-sensitive K^+ flux is 1.4 between 0 and 10 °C and rises to approximately 2 between 10 °C and 20 °C (Raynard and Cossins, 1991). For the Na^+/K^+ pump, Q_{10} values of 1.7 (between 10 °C and 20 °C) and 3.3 (between 0 °C and 10 °C) have been reported (Raynard and Cossins, 1991).

While the acute Q_{10} matches needs during acute temperature changes, it is evident that there are interspecific differences in P_{Cl} that correlate with life under different temperature regimes. At a given constant temperature, the P_{Cl} values of trout, turtle and human RBCs differ (Fig. 2), but if the data are compared at preferred body temperatures, the situation is different. The preferred body temperature under normoxic conditions is 16 °C in rainbow trout (Schurmann et al., 1991), 26–30 °C in turtle (Hammond et al., 1988) and 37 °C in man. At these respective temperatures, the P_{Cl} values of the RBCs are remarkably similar in the three species.

This idea can be elaborated by including the P_{Cl} data obtained using the same technique on other animals (Fig. 3). The two endothermic animals, man and chicken, show strong temperature-dependence, but P_{Cl} is high at physiological temperature. P_{Cl} values in ectothermic animals (four species of fish and two species of reptile) lie within a relatively narrow band around the physiological values in the endotherms (Fig. 3, green band). The band narrows down even further using P_{Cl} at preferred body temperatures (Fig. 3). Cod (*Gadus morhua*) has a preferred temperature of 14 °C (Schurmann and Steffensen, 1992), similar to the preferred temperature of 16 °C in trout, and P_{Cl} values are remarkably similar (Fig. 3; Jensen and Brahm, 1995). Eel (*Anguilla anguilla*) has the lowest P_{Cl} at 15 °C (Fig. 3; Jensen and Brahm, 1995), but its preferred temperature is 17–20 °C (Haro, 1991), slightly elevating P_{Cl} (assuming a Q_{10} as in trout). In carp (*Cyprinus carpio*), the final temperature preferendum is 32 °C (Pitt et al., 1956), and an extrapolation of the measured P_{Cl} from 15 to 32 °C

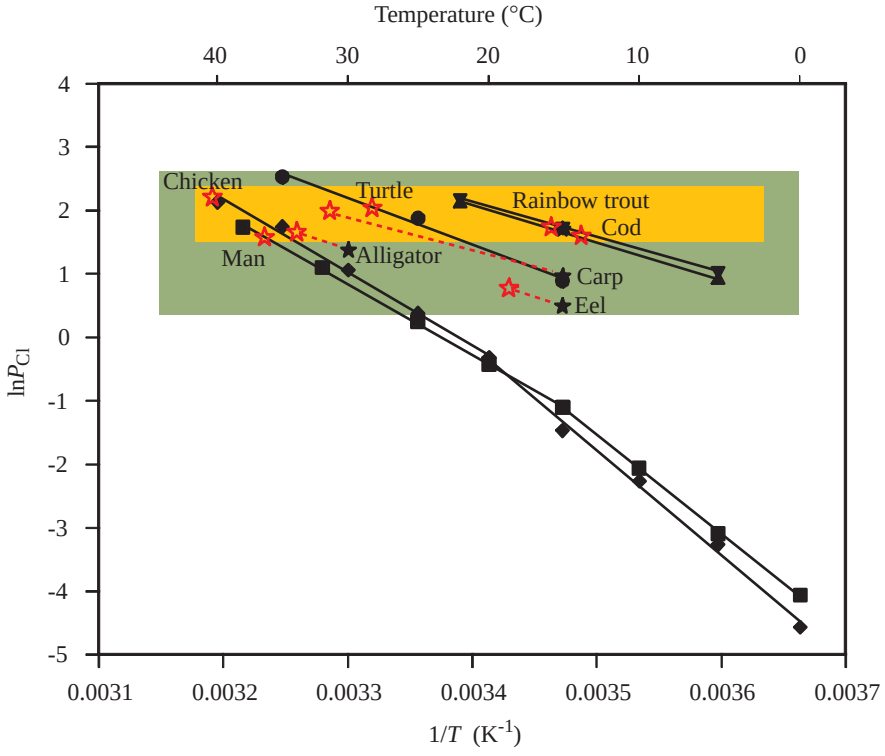

Fig. 3. Overview of the erythrocyte Cl^- permeability in various ectothermic (turtle, alligator, rainbow trout, eel, carp, cod) and endothermic (man, chicken) animals. The data are shown as Arrhenius plots ($\ln P_{Cl}$ *versus* $1/T$, where T is absolute temperature), with the original P_{Cl} values ($\mu m\,s^{-1}$). Filled symbols refer to measured values, and open red stars show values extrapolated to the preferred body temperature of each species. All measured data were collected using the continuous flow tube method. See text for further details. Data were obtained from the following sources: present study; Brahm, 1977; Brahm and Wieth, 1977; Jensen and Brahm, 1995; Jensen et al., 1998. The green band encompasses measured values in ectotherms; the orange band highlights the similar P_{Cl} at preferred body temperatures. ■, man; ◆, chicken; ●, turtle; ▼, ▲, rainbow trout; ★, alligator; ★, cod; ★, carp; ★, eel.

(assuming a Q_{10} as in trout) brings the P_{Cl} value close to that in man and chicken (Fig. 3). Similarly, extrapolation of P_{Cl} in the alligator (*Alligator mississippiensis*) (assuming a Q_{10} as in turtle) from 30 °C (Jensen et al., 1998) to the preferred body temperature of 32–35 °C (Colbert et al., 1946) produces a value akin to that in man (Fig. 3). Thus, the P_{Cl} values of ectothermic and endothermic animals are remarkable similar when compared at the preferred temperatures of the species. The eel is known to have an exceptionally low rate of Cl^- transport across its gills (e.g. Hyde and Perry, 1989), and the P_{Cl} in eel RBCs is also somewhat reduced (Fig. 3). Accepting the eel as a negative variant, P_{Cl} values become almost identical at the preferred body temperatures (Fig. 3, orange band).

The data in Fig. 3 show that there is a 'right shift' of the $\ln P_{Cl}$ *versus* $1/T$ (where T is absolute temperature) curves in ectotherms compared with endotherms to an extent that depends on the temperature regime encountered by a given species. It would be interesting to elaborate further on this idea by measuring P_{Cl} in Arctic and Antarctic fish that may be hypothesised to have $\ln P_{Cl}$ *versus* $1/T$ curves further to the right of that of rainbow trout. The shift of $\ln P_{Cl}$ *versus* $1/T$ curves according to thermal niche suggests an appropriate evolutionary adaptation of anion exchange function. It is difficult to comprehend this adaptation fully, because metabolic rate, haematocrit and other variables that set the stage for CO_2 transport vary between the different endothermic and ectothermic animals. Capillary transit time, however, is an essential parameter for RBC anion exchange *in vivo*. One possible interpretation of the data is, therefore, that capillary transit times could be similar among the animals, and that the relatively constant P_{Cl} at preferred body temperatures reflects an adaptation that secures virtual completion of RBC anion exchange during capillary passage in the normal resting steady state.

The interspecific difference in thermal sensitivity for AE1-mediated Cl^- transport that is evident from the present data resembles the species variation in thermal sensitivity according to adaptation temperature that applies to some other proteins. One example is provided by orthologous homologues of muscle-type lactate dehydrogenase (LDH). The K_m values of pyruvate in species adapted to different thermal environments are similar when measured at the respective physiological temperatures (Somero, 1996). Furthermore, the LDHs of eurythermal species have relatively flat K_m *versus* temperature curves, whereas the LDHs of stenothermal species tend to be very temperature-sensitive, with appropriate K_m values only within the narrow temperature range encountered by the species (Somero, 1996). Such interspecific differences in protein function need only involve very minor structural differences (e.g. substitution of one or a few amino acid residues) between protein homologues (Somero, 1996).

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