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BICARBONATE/CHLORIDE TRANSPORT KINETICS AT 37°C AND ITS RELATIONSHIP TO MEMBRANE LIPIDS IN MAMMALIAN ERYTHROCYTES

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The rate of exchange of HCO_3^- with Cl^- at 37°C in erythrocytes of ten mammalian species was studied. The rate constant increases from 7 s^{-1} (ox) to 16 s^{-1} (rat), and is inversely proportional to the body size (log_{10}) of the species. It is found that the membrane permeability in different species is positively correlated to the red cell membrane phosphatidylcholine or arachidonate content, and is negatively correlated to the sphingomyelin or linoleate content.

Key words: Cl^- transport; Membrane lipid; Transport kinetics; (Mammalian erythrocyte)

Introduction

Mammals of different body size show differences in their metabolic rates. The rate of oxygen consumption per gram of body mass of small mammals is higher than that of large mammals [1], and also is the CO_2 production. How the faster release of CO_2 in smaller animals is accomplished in the cellular level is an interesting problem in comparative physiology. Carbonic anhydrase activity in mammalian blood has indeed been shown to be related negatively to the body size [2]. However, this enzyme exists in amounts far exceeding that needed for CO_2 hydration-dehydration in the erythrocytes, so that a few-fold change in activity may be of little significance to the CO_2 elimination rate. Another process involved in the CO_2 transport scheme in blood, the Cl^- shift or the HCO_3^--Cl^- exchange, has not been studied for different mammals at the physiological temperature. HCO_3^--Cl^- exchange across the red cell membrane helps the rapid establishment of the HCO_3^- pool in the plasma during the capillary transit time when blood passes through tissue capillaries. It is responsible for the removal of about 40% of the CO_2 eliminated from blood (calculated from Ref. 3). Its inhibition can lead to a decrease in the rate of CO_2 production [4]. HCO_3^--Cl^- exchange is a fast process. The half-time is about 0.1 s in human red blood cells at 37°C [5,6]. Usually rapid mixing techniques have to be used for its study. The kinetically equivalent Cl^--Cl^- self-exchange at low temperature has been studied extensively using radioactive tracer [7,8]. The apparent activation energy of both the HCO_3^--Cl^- exchange and the Cl^- self-exchange in human red cells at low temperatures is different from that at physiological temperature [5,9–11], and there are evidences of a phase transition occurring at around 18°C in the human red cell membrane [12,13]. It is therefore necessary to study the transport at 37°C to explore its physiological significance. In this report, we will present the results on the study of the red cell HCO_3^--Cl^- transport kinetics at 37°C in ten common mammalian species. The relationship between the transport rate and the red cell membrane lipid

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composition of the ten species will be discussed. An abstract of this work was presented in the 7th International Biophysics Congress in Mexico City [14].

Materials and Methods

Blood from mature laboratory animals was obtained by decapitation (mouse, rat, guinea pig), or by venipuncture from foreleg vein (dog), jugular vein (goat, pig) or ear vein (rabbit). Ox and some pig blood were obtained from local slaughter houses. Blood of cat was obtained by heart puncture in one case and from cannulated femoral artery from cats anesthetized with ether in other cases.

Heparinized blood red cells were washed three times in isotonic saline (150 mM NaCl). Cells from two to seven animals were sometimes pooled together depending on the size of the samples. The preparation of solutions, the procedure of the experiment and the analysis of data have been described before [5,15,11], except that the intracellular pH of the cell suspensions were calculated from the measured extracellular pH according to the data of Funder and Wieth [16]. Red cell suspension of 15% hematocrit and a pH of about 8, containing 2 mM $NaHCO_3$ and 600 Wilbur-Anderson units/ml of bovine carbonic anhydrase was mixed in a stopped-flow glass pH electrode rapid reaction apparatus [17,5] with a 30 mM phosphate-buffered saline (pH 6.7) at 37°C. The rate of extracellular pH change measured gives the HCO_3^- flux across the red cell membrane, which then gives a measure of the rate constant k and the apparent permeability P for the HCO_3^-/Cl^- movement [11]. Q_{10} was obtained by taking the ratio of the measured rate constant at 37°C to that at 27°C.

Results and Discussions

The rate constants k of the exchange of HCO_3^- with Cl^- at 37°C were plotted in Fig. 1 against the body weight of the population of the ten mammalian species in a logarithmic scale. There is a significant negative linear correlation between k and $\log_{10}$ of the body weight ($r = -0.670$, $P < 0.05$) though the result from guinea pig falls off

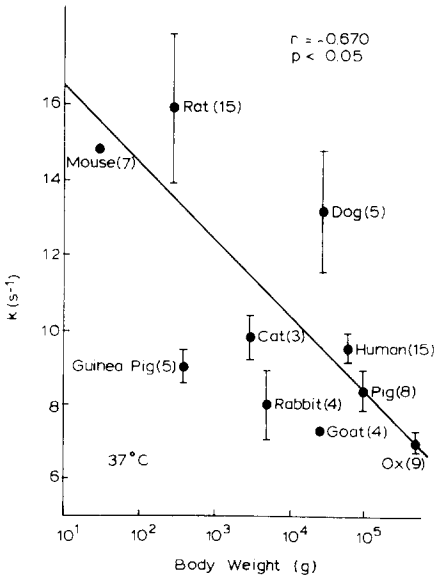

Fig. 1. The red cell HCO_3^--Cl^- exchange rate constants at 37°C in ten mammalian species. The exchange rate constant k at 37°C was measured by using a stopped-flow rapid reaction apparatus with a glass pH electrode attached.
$k = (dpH/dt) \cdot B \cdot (1 - Hct)([HCO_3^-]_i - [HCO_3^-]_o)^{-1}$
$\cdot Hct^{-1}\alpha^{-1}$, where dpH/dt is the rate of pH change measured, B is the buffer power of the extracellular fluid, Hct is the hematocrit in the reaction mixture, α is the cell water fraction, and $[HCO_3^-]_i$, $[HCO_3^-]_o$ are the intra- and extracellular HCO_3^- concentration respectively. $\alpha = 0.7$ is used for all species (unpublished observation). Body weight of each species is presented as the average body weight of the population. Vertical bars express standard errors. The numbers after the species names indicate the numbers of animals studied.

the line. Small mammals such as rat or mouse do show higher rate of HCO_3^--Cl^- exchange which will allow a faster establishment of the HCO_3^- pool in the plasma during the capillary transit time when blood flows through the tissue capillaries. Therefore smaller animals probably accomplish higher rate of CO_2 production partially through a faster exchange of HCO_3^- for Cl^- across their red cell membranes. Q_{10} for the exchange for temperatures between 37 and 27°C ranges from 1.4 (goat) to 2.2 (rat) in the sequence goat $\doteq$ ox $\doteq$ guinea pig < human (1.7) < dog $\doteq$ rat.

Fig. 2 compares our data at 37°C with the rate of Cl^- self-exchange at 0°C as reported by Wieth et al. [8]. There is a linear correlation between the two sets of data ($r = 0.696$, $p < 0.05$) although one might expect a better correlation. The rate at 37°C

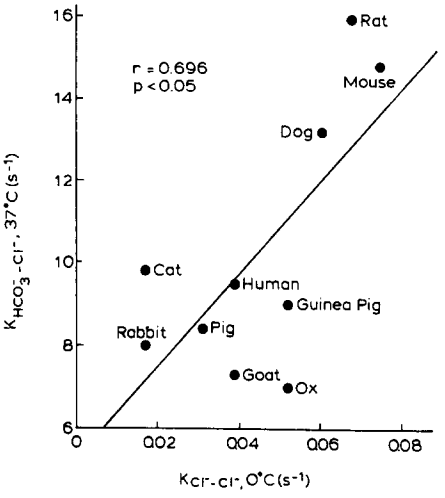

Fig. 2. Comparison between our data and the data for Cl^- self-exchange at 0°C as reported by Wieth et al. [8].

is about 100-fold of that at 0°C for most of the species. Ox and goat red cells which transport HCO_3^- and Cl^- slower than human red cells at 37°C transport faster at 0°C than human cells. It needs further clarification whether this implies that ox and goat red cell anion transport systems respond to temperature change differently from humans' such that the turnover number of the carrier protein decreases with decreasing temperature more in human red cells than in ox and goat red cells. There is no experimental evidence so far that the carrier proteins [18] associated with the anion transport in red cells are structurally different in various mammalian species. Rather, they often exhibit many common properties [19]. Dog erythrocytes transport HCO_3^- and/or Cl^- faster than human or cat erythrocytes at both 37°C and 0°C. However, the number or density of anion transport sites per cell in dog erythrocytes as estimated by H_2DIDS (dihydro-4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid) binding is fewer than that in cat or human erythrocytes [19]. Therefore, it seems that the rate of HCO_3^-/Cl^- transport cannot be correlated positively with the number or density of anion transport sites in mammalian erythrocytes, at least for dog, cat and human. Perhaps the difference in rate is due to a difference in the turnover number of the carrier protein in various mammalian erythrocyte membranes as is suggested in Ref. 19. Our data in HCO_3^--Cl^- exchange are compared with data on phosphate transport [20] in Fig. 3. Rabbit and guinea pig red cells exchange HCO_3^- with Cl^- slower than human red cells, but transport phosphate faster. This suggests that the mechanism of phosphate transport may differ from that of HCO_3^-/Cl^- transport although presumably the same carrier protein (band 3) [18] is involved. Passow and Lepke [21] showed that dansylation of the red cell membrane affected sulfate transport and Cl^- transport differently.

We tried to correlate our measured membrane HCO_3^-/Cl^- permeability with the red cell membrane lipid composition of the species reported in the literature [22,23]. There is no correlation between the permeability and the content of cholesterol, glycolipid, ganglioside, or phospholipid in total lipids. However when distribution pattern of single phospholipids is considered, similar to phosphate transfer [20], the HCO_3^-/Cl^- permeability is found to correlate positively with the percentage of phosphatidylcholine in total membrane phospholipids and negatively with that of sphingomyelin (Fig. 4). The fatty acid composition of the mammalian red cell membranes varies [23]. The HCO_3^-/Cl^- permeability shows positive linear correlation with the percentage of arachidonic acid (20:4) in total fatty acids, and negative linear correlation with that of linoleic acid (18:2) in

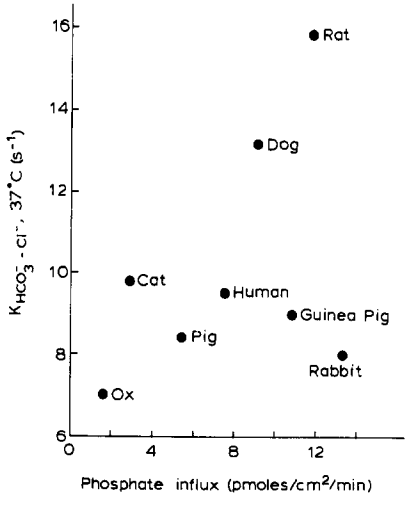

Fig. 3. Comparison between the transport rate of HCO_3^-/Cl^- at 37°C and the phosphate transfer reported by Gruber and Deuticke [20].

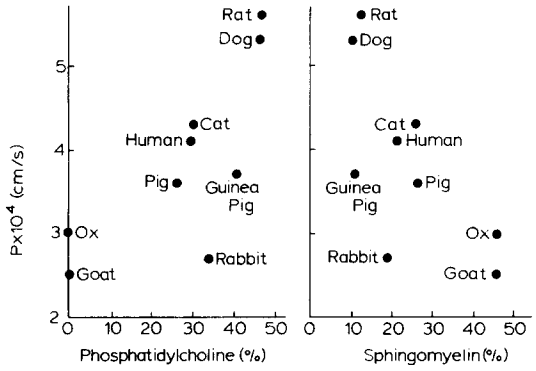

Fig. 4. Relationship between the HCO_3^-/Cl^- permeability of red cell membranes and the percentage of phosphatidylcholine and sphingomyelin in total red cell membrane phospholipids (as weight percent phosphorus) in different mammalian species [22]. Permeability $P = k \cdot \alpha \cdot v/a$, where v/a is the volume to surface area ratio of one red blood cell of the individual species. The v/a (μ^3/μ^2) values used are 61/121, 77/129, 48/83, 57/118, 67/117, 25/50, 87/142, 58/95, and 58/95, for rat, guinea pig, cat, rabbit, dog, goat, human, pig and ox, respectively (adopted from Ref. 20, except the area for the goat red cell which was from Ref. 24 assuming a cylindrical shape).

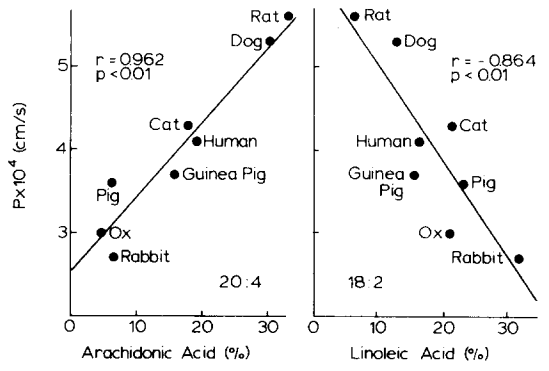

Fig. 5. HCO_3^-/Cl^- permeability of mammalian red cell membranes shows positive linear correlation with the percentage of arachidonic acid and negative linear correlation with the percentage of linoleic acid in total red cell membrane fatty acids in different species [23].

Fig. 5. The hydroxyl group and the amide bond in the sphingosine group of the sphingomyelin molecules allow strong hydrogen bond formation between these groups and other phospholipids, cholesterol and proteins in the external surface of the red cell membrane [25]. The strong 'hydrogen belt' suppresses the permeability of artificial phospholipid membranes [26]. This fact can probably account for the lower transport rate shown in Fig. 4 in mammalian erythrocyte with higher content of sphingomylin in the membrane. In artificial phospholipid membrane systems and microorganisms, it has been shown that permeability increases with rising number of double bonds in the fatty acid chains [27,28], probably due to a wider space between the fatty acid chains in the inner lipid layer and a more fluidic state of the membrane. This seems to be consistent with our observation in Fig. 5 that transport rate increases with increasing content of arachidonic acid (20:4) in the membrane. Anion transport across the red cell membrane is a passive transport mediated by the carrier protein 'band 3'. Our data seem to suggest that the transport mediated by a protein is affected in the same way as simple diffusion by the lipid microenvironment of the membrane. It has been mentioned above that the HCO_3^-/Cl^- transport rate can not be correlated with the density of the anion transport sites and the difference in rate is probably due to a difference in turnover numbers of the carrier protein in various mammalian red cell membranes. This leads one to speculate that the turnover number of the carrier protein may be affected by the lipid microenvironment probably through lipid-protein interactions. Direct proof for a dependency of protein-mediated transport rate on the membrane lipid composition would require the study of the same kind of erythrocytes with altered content of only one lipid component. This has been attempted in vitro [29] recently.

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