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KINETIC CHARACTERISTICS OF BICARBONATE-CHLORIDE EXCHANGE ACROSS THE NEONATAL HUMAN RED CELL MEMBRANE

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The kinetics of HCO_3^-/Cl^- exchange across red cell membrane of newborn infants was studied using a stopped-flow rapid reaction apparatus with a glass pH electrode attached. The measured apparent permeability P is $(1.35 \pm 0.08$ (S.E.)$) \cdot 10^{-4}$ cm/s ($n=30$) for newborns, compared with $(3.1 \pm 0.4) \cdot 10^{-4}$ cm/s ($n=15$) for adults. These correspond to half-times of 0.2 s for newborns and 0.1 s for adults indicating that neonatal red cells exchange Cl^- for HCO_3^- only half as fast as do adult cells. The temperature dependence of the exchange rate was studied from 2 to 42°C. From the Arrhenius plot the activation energy of the exchange process in neonatal red cells changes from 22.9 kcal/mol (low temperature) to 4.8 kcal/mol (physiological temperature) at a transition temperature of 17°C. These values are lower than the corresponding values for adult red cells, 34.7 and 10.2 kcal/mol. HCO_3^-/Cl^- exchanges in both adult and neonatal red cells are inhibited by phlorizin. Inhibition constants K_i are 0.8 mM and 2.5 mM for adults and newborns, respectively. The differences in the values of the HCO_3^-/Cl^- exchange rate constant and the activation energy of the exchange process between neonatal and adult red cells indicate that there is a modification of HCO_3^-/Cl^- transport system in the neonatal red cell membranes.

The exchange of HCO_3^- with Cl^- across the red blood cell membrane (Cl^- shift) during and after CO_2 outflow in the lungs or uptake in the tissues, is an important step in the CO_2 elimination processes in respiration. The rate of this exchange process might limit the amount of CO_2 which can be eliminated from blood or tissue during capillary transit time. Pulmonary transit time has been estimated to range from 0.1 to 2 s [1–3]. The exchange of HCO_3^- with Cl^- across the red cell membrane is a fast process. Its study usually requires rapid mixing apparatuses such as stopped-flow or continuous-flow rapid reaction apparatuses. The half-times of HCO_3^-/Cl^- exchanges across the adult human red blood cell membrane at 37°C have been reported to range from 0.04 to 0.2 s [4–9].

Neonatal human red cells have transport properties quite different from adult red cells. The former have decreased active K^+ influx [10], decreased phosphate uptake [11] and lower water permeability [12]. The HCO_3^-/Cl^- exchange of the neonatal red cells is also slower than that of the adult ones [13]. In this article, we report the results of more extensive study on the kinetics of HCO_3^-/Cl^- exchange of neonatal red cells, the temperature dependence and the effect of phlorizin inhibition. Effort has been made to compare these cells with the adult red cells.

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Materials and Methods

All solutions were prepared from grade GR or EP Chemicals produced by E. Merck Co., G.F.R., unless otherwise stated. Methods of preparation of all solutions, the procedure of experiment, the calculation and analysis of data were the same as previously reported [5,13].

Fresh mixed venous and arterial umbilical cord blood of full-term infants was obtained from the delivery room of the Hospital of National Taiwan University or the Maternity and Infant Care Center of Taiwan Provincial Nursing School. Fresh adult venous blood was obtained from hematologically healthy blood sellers at the Hospital of National Taiwan University or healthy young college graduates. The washed red cells were suspended in a medium of 146.5 mM NaCl and 3.5 mM KCl contained in a glass syringe and gave a hematocrit of about 15%. $NaHCO_3$, bovine carbonic anhydrase (carbonate dehydratase, carbonate hydro-lyase, EC 4.2.1.1, Sigma Chemical Co., St. Louis, MO, No. C-7500) and NaOH were added so that the final concentrations in the suspension were 2 mM $NaHCO_3$ and 600 Wilbur-Anderson units/ml of carbonic anhydrase, and the pH was about 8. The suspension was mixed in a stopped-flow glass pH electrode rapid reaction apparatus [5,14] with a pH 6.7 buffer solution of 15 mM Na_2HPO_4, 15 mM KH_2PO_4 and 112.5 mM NaCl. The change of pH with time in the reaction mixture was monitored. This extracellular pH change is rate-limited by the exchange of HCO_3^- for Cl^- across the red cell membrane under these experimental conditions* [5]. The rate of extracellular pH change is therefore a measure of the speed of the HCO_3^-/Cl^- exchange.

In the study of temperature dependence, the cell suspension and the buffer solution were equilibrated to the specific temperature studied before mixing, and all measurements were done at that temperature. At 2°C, the composition of the pH 6.7 buffer solution was changed to 5 mM Na_2HPO_4, 5 mM KH_2PO_4, 137.5 mM NaCl. The buffer capacity is lower in order to speed up the pH change.

When phlorizin inhibition was studied, phlorizin (Sigma Chemical Co., St. Louis, MO, No. P3377) of known concentration was dissolved in both the suspending medium of the red cell and the pH 6.7 buffer solution at about 40°C. The mixing experiment was done at 37°C.

The initial flux J of HCO_3^- out of the red cell per unit membrane surface area was determined from the initial dpH/dt observed in the reaction mixture right after the flow stopped in the stopped flow rapid reaction apparatus:

$$J = B_o\,(dpH/dt)(1 - Hct)/(Hct \times a/v)$$

where B_o is the buffer capacity of the extracellular fluid of the reaction mixture, Hct is the hematocrit of the reaction mixture and a and v are the area and volume per cell, respectively. For adult red cells 142 μm^2 (calculated from Ref. 18), and 87 μm^3 [19] were used for a and v, respectively. For neonatal red cells, 187 μm^2 and 106 μm^3 [20,12] were used for a and v, respectively. The intracellular and extracellular HCO_3^- concentrations immediately after the flow stopped were computed from the measured extracellular pH at that time using the equilibrium relation for CO_2 hydration-dehydration reaction with the assumption that CO_2 concentrations are the same intra- and extracellularly, and total CO_2 content remains constant. The equations, given previously [5] were solved with an interative procedure. An apparent permeability for the HCO_3^-/Cl^- movement was then calculated as

$$P = J/([HCO_3^-]_i - [HCO_3^-]_o)$$

This apparent permeability normalizes the fluxes for small differences in $[HCO_3^-]$ gradient and is related to the previously defined [5] rate constant for HCO_3^-/Cl^- exchange $k = 0.693/t_{1/2}$ and cell water fraction α(v/v) by the relation

$$P = k \times \alpha \times v/a$$

* Intracellular carbonic anhydrase speeds up CO_2 hydration-dehydration reaction by 10000- to 13000-fold in adult red cells [15,16]. The enzymatic activity in neonatal red cells is about one fourth of that in the adult ones [17], which will speed up the CO_2 reaction by 2000-fold. This corresponds to a half-time for the intracellular CO_2 hydration reaction of the order of milliseconds. So intracellular CO_2 hydration cannot limit the rate of pH change. Therefore, this conclusion on rate limitation drawn from the results of adult red cells [5] remains valid.

Results

A typical experimental record for the measurement of kinetics of HCO_3^-/Cl^- exchange in neonatal red cells is reproduced in Fig. 1. The top three traces indicate the pH of the fluid in the measuring chamber as a function of time. The bottom trace indicates the linear flow speed through the mixing chamber. An upward deflection represents an increase of flow rate. Before flow starts, phosphate-buffered saline is in the measuring chamber. During and after flow, extracellular pH of the reaction mixture increases towards its equilibrium value as CO_2 enters the cells effecting the movement of H^+ from extra- to intracellular space. From the slope at time zero, dpH/dt, the initial HCO_3^- flux J and the apparent permeability P are then determined.

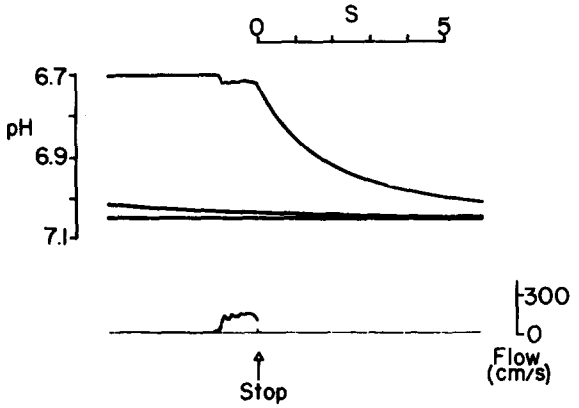

Fig. 1. Experimental record of the measurement of kinetics of HCO_3^-/Cl^- exchange across the neonatal red cell membrane at 37°C. Suspension A: washed cord blood red cells. Hematocrit 0.15, 2 mM $NaHCO_3$, 600 U/ml carbonic anhydrase, pH 8.07. Suspending medium: 146.5 mM NaCl, 3.5 mM KCl, CO_2 removed. Solution B: 30 mM phosphate buffer, 112.5 mM NaCl, CO_2 removed, pH 6.7. Suspension A and solution B were mixed in equal volumes in a stopped-flow rapid reaction apparatus with a glass pH electrode attached.

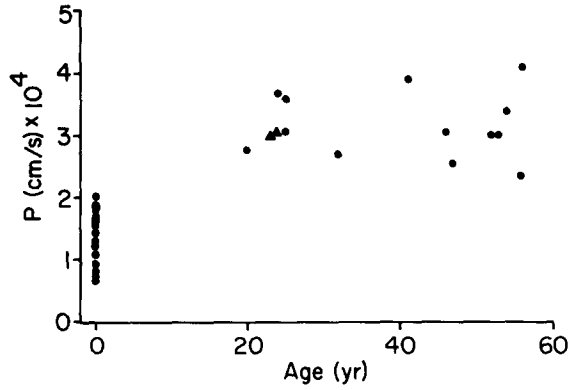

Fig. 2. Apparent permeabilities for the HCO_3^-/Cl^- exchange across the red blood cell membrane of newborns and adults. ▲ represents data obtained from female donors. $P=J/([HCO_3^-]_i - [HCO_3^-]_o)$. The HCO_3^- flux J is determined from the experimental record by the relation $J = B_o\ (dpH/dt)(1 - Hct)/(Hct \times a/v)$ where B_o is the buffer capacity of the extracellular fluid of the reaction mixture. Hct is the hematocrit and a and v are the area and volume per cell, respectively.

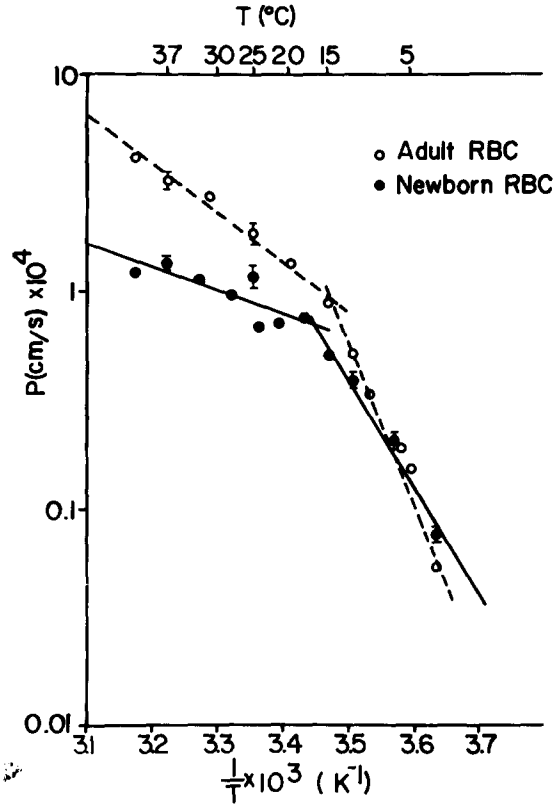

Fig. 3. Arrhenius plot for the apparent permeabilities for HCO_3^-/Cl^- exchange across red cell membrane. Comparison between newborns and adults. The correlation coefficients of the linear regression lines fitting the adult data are 0.991 and 0.992 for the low and physiological temperature ranges, respectively. The corresponding values for the newborns are 0.989 and 0.800, respectively.

Apparent permeabilities of HCO_3^-/Cl^- exchange across red cell membrane of newborns and adults from age 20 to 56 were obtained at 37°C and at an intracellular pH of 7.6 ± 0.2 and extracellular pH of 6.70 ± 0.07. The values are plotted

TABLE I

THE EFFECT OF TEMPERATURE ON THE RATE OF HCO_3^-/Cl^- EXCHANGE ACROSS THE MEMBRANE OF NEONATAL HUMAN ERYTHROCYTES

Donor	Temp. (°C)	$J \pm$S.E. (n) (nmol/cm^2 per s)	$[HCO_3^-]_i$ (mM)	$[HCO_3^-]_o$ (mM)	k (s^{-1})	$P(\times 10^4)$ (cm/s)
CK	2	0.0196 ± 0.0008 (4)	3.39	0.72	0.183 ± 0.007	0.075 ± 0.003
CM		0.0276 ± 0.0005 (4)	4.00	0.70	(n=3)	(n=3)
CM		0.020 ± 0.003 (6)	4.77	0.62		
CO	7	0.100 ± 0.012 (3)	4.79	0.60	0.50 ± 0.06	0.20 ± 0.02
CP		0.0943 ± 0.0005 (2)	5.77	0.60	(n=2)	(n=2)
CL	12	0.16 ± 0.01 (6)	5.14	0.62	0.93 ± 0.06	0.38 ± 0.02
CK		0.12 ± 0.02 (6)	3.70	0.68	(n=3)	(n=3)
CN		0.15 ± 0.01 (5)	4.25	0.68		
CS	15	0.164 ± 0.001 (3)	3.85	0.69	1.22	0.498
CO	18	0.36 ± 0.02 (5)	5.11	0.59	1.86	0.759
CJ	21.5	0.297 ± 0.003 (7)	4.62	0.62	1.74	0.710
CC	24	0.39 ± 0.04 (3)	5.96	0.56	1.68	0.686
CD	25	0.43 ± 0.02 (4)	5.13	0.60	2.87 ± 0.36	1.2 ± 0.1
CE		0.52 ± 0.03 (6)	4.70	0.64	(n=3)	(n=3)
CN		0.43 ± 0.02 (4)	3.59	0.69		
CB	28	0.49 ± 0.02 (4)	5.49	0.56	2.34	0.955
CI	32.5	0.51 ± 0.04 (4)	4.95	0.64	2.79	1.139
CC	37	0.56 ± 0.02 (3)	4.07	0.67	3.3 ± 0.2	1.35 ± 0.08
CD		0.68 ± 0.04 (4)	4.62	0.64	(n=30)	(n=30)
CE		0.84 ± 0.04 (4)	4.95	0.66		
...						
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CH		0.63 ± 0.03 (4)	6.77	0.56		
CH	42	0.71 ± 0.02 (4)	6.29	0.59	2.96 ± 0.06	1.21 ± 0.02
CJ		0.56 ± 0.04 (5)	4.99	0.63	(n=2)	(n=2)

Cell suspension A: washed cord blood red cells. Hematocrit 0.16, 2 mM $NaHCO_3$, 600 U/ml carbonic anhydrase, pH 8.0 ± 0.2. Suspending medium: 146.5 mM NaCl, 3.5 mM KCl, CO_2 removed. Solution B: 30 mM phosphate buffer, 112.5 mM NaCl, or for 2°C, 10 mM phosphate buffer, 137.5 mM NaCl, CO_2 removed, pH 6.8 ± 0.1. Suspension A and solution B were mixed in equal volumes at the specific temperature in a stopped-flow rapid mixing apparatus with a glass pH electrode attached. $J \pm$S.E. is the HCO_3^- flux determined. $[HCO_3^-]_i$, $[HCO_3^-]_o$, k, and P are calculated intra- and extracellular $[HCO_3^-]$, rate constant, and apparent permeability, respectively. Only four out of 30 data obtained at 37°C are shown.

versus age in years in Fig. 2. For adults, there is no significant linear correlation between P and age in the range we studied ($r = 0.038$). The average P value is $(3.1 \pm 0.4$ (S.E.)$) \cdot 10^{-4}$ cm/s ($n = 15$). The average P value for newborns is $(1.35 \pm 0.08) \cdot 10^{-4}$ cm/s ($n = 30$), which is significantly lower than that for adults (Student's t-test: $p < 0.001$).

The HCO_3^-/Cl^- exchange rate depends on temperature. The experimental data are presented in Table I and Fig. 3. In Fig. 3, the data for neonatal red cells are plotted as the $\log_{10}$ of the apparent permeability P against $1/T$, where T is the absolute temperature (Arrhenius plot). Data for adult red cells determined previously [5] are plotted together for comparison. When the temperature increases from 2 to 37°C, the apparent permeability P of the HCO_3^-/Cl^- exchange in neonatal red cells increases from $(0.075 \pm 0.003) \cdot 10^{-4}$ cm/s ($n = 3$) to $(1.35 \pm 0.08) \cdot 10^{-4}$ cm/s ($n = 30$). The change is about 20-fold for newborns, while the change for adults in the same temperature range is 60-fold. The data are better

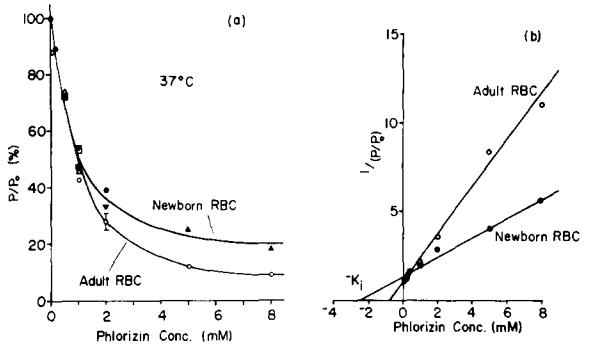

Fig. 4. (a) Phlorizin inhibition on the rate of HCO_3^-/Cl^- exchange across the red cell membrane. Closed symbols are data for newborn red blood cells and open symbols are data for adult red blood cells. Different symbols indicate different individuals. (b) Dixon plot of phlorizin inhibition. For adults, the regression line intercepts the absissa at $-K_i = -(0.8 \pm 0.3$ (S.E.)) mM, $r = 0.995$. For newborns, $K_i = (2.5 \pm 0.5$ (S.E.)) mM, $r = 0.990$.

fitted by two straight lines instead of one single line. Linear regression lines fitting the data with the largest difference of slopes were shown in Fig. 3. This kind of analysis yields the largest change in Arrhenius activation energies. For adults, the activation energy of the exchange process changes from 34.7 kcal/mol (low temperature) to 10.2 kcal/mol (physiological temperature) at a transition temperature at 15°C. For newborns, the corresponding change is from 22.9 kcal/mol to 4.8 kcal/mol at a transition temperature of 17°C.

The effect of phlorizin on the HCO_3^-/Cl^- exchange rate of neonatal and adult red cells is shown in Fig. 4. The extracellular and intracellular pH during the measurements were maintained at 6.77 ± 0.01 and 7.6 ± 0.1, respectively. In both kinds of cells the apparent permeability expressed as percentage of the uninhibited value decreases as the phlorizin concentration in the mixture increases (Fig. 4a). The concentration of phlorizin required for 50% inhibition for both kinds of cells is 1 mM. If noncompetitive inhibition is assumed [21], a Dixon plot of the data (Fig. 4b) gives an inhibition constant K_i of 0.8 ± 0.3 (S.E.) mM for adults and 2.5 ± 0.5 mM for newborns. The two values are very close.

Discussion

Data in Fig. 2 show that under the same experimental conditions, the neonatal red cell membranes exchange Cl^- for HCO_3^- only half as fast as do red cell membranes of adults. While neonatal red cells are considerably larger than adult ones, the a/v ratios for the two types of cells are different by only 5%, presumably because they both have the biconcave shape. The rate of exchange of HCO_3^- with Cl^- in the red cells as determined by

$$k = P \times (1/\alpha) \times (a/v)$$

therefore also manifests a 2-fold difference. The intracellular water contents of the two types of cells are both 0.7 (unpublished result). The average exchange rate constant for newborns at 37°C is 3.3 ± 0.2 (S.E.) s^{-1}, corresponding to an average half-time of about 0.2 s for the process, while the adult value is 0.1 s. This fact suggests that the process of taking up CO_2 by plasma or eliminating CO_2 from plasma is slower or less efficient in newborn infants when blood flows by tissue cells or alveoli. Computer simulation showed that a reduction of the red cell membrane permeability for HCO_3^- and Cl^- can lead to a reduction in pulmonary CO_2 elimination [22]. If newborn infants have the same or shorter capillary transit time as adults, their slower red cell HCO_3^-/Cl^- exchange rate could partially limit CO_2 elimination from blood in the lung, and thus decrease the efficiency of total CO_2 exchange in newborn infants. The capillary transit time in newborns is not known. However newborn infants have a higher pulse rate [23] and larger blood volume per unit weight [24] than adults, and their lung morphology is different [25]. It is therefore possible that the neonatal capillary transit time is different from the adult value.

The Arrhenius plot (Fig. 3) of the temperature dependence of HCO_3^-/Cl^- exchange rate in neonatal red cells is similar to that in adult ones. Both have low Arrhenius activation energies in the physiological temperature range and high Arrhenius activation energies in the low temperature range. And the transition temperature is around 15 to 17°C. This is consistent with the work on Cl^- self-exchange by Brahm [4] and HCO_3^-/Cl^- exchange by Obaid and Crandall [26]. Both groups reported for adult red cells a decrease of activation energy with temperature increasing from 0 to 37°C at a transition tempera-

ture around 15–17°C. No data on neonatal red cells are available for comparison. A change of activation energy for the transport process around 15–17°C may imply a phase transition occurring around this temperature in the red cell membranes.

The absolute values of the activation energy for the HCO_3^-/Cl^- exchange in both temperature ranges calculated from Fig. 3 are lower in the neonatal red cells than in the adult ones. The value for the neonatal red cells is only about half of that for the adult ones in the physiological temperature range. This suggests that HCO_3^-/Cl^- exchange across the neonatal red cell membrane encounters a lower energy barrier at all temperatures investigated, and the difference is more pronounced in the physiological temperature range. The inhibition constant K_i of 0.8 mM for the inhibition of phlorizin on the HCO_3^-/Cl^- exchange in adult red cells reported here is very close to the value 0.6 mM reported by Schnell [21] on the sulfate exchange. Our data showed that neonatal red cells have almost the same phlorizin inhibition constant as the adult ones. This suggests that phlorizin may have the same apparent affinity for the HCO_3^-/Cl^- transport system in the neonatal red cell membrane as that in the adult one.

Differences in transport properties are manifestations of different membrane structural properties. The differences in the HCO_3^-/Cl^- exchange rate and the activation energy of exchange between the neonatal and the adult red cells indicate that there are certain modifications in the chemical structure and/or conformation of the HCO_3^-/Cl^- transport system in the neonatal red cell membranes. No qualitative or gross quantitative difference was found in the sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of membrane proteins of neonatal and adult red cells [27,28]. The decreased rate of exchange in neonatal red cells may imply that the band 3 protein associated with anion transport functions in the adult red cell membranes [29,30] exists in decreased amount in the neonatal red cell membrane. Although the finding that inhibition constants of phlorizin for the two kinds of cells have similar magnitude seems to be consistent with this implication, the former cannot be taken as the support for the latter because the exact mechanism of phlorizin inhibition is still unknown. Furthermore, since band 3 contains a heterogeneous population of proteins, it is quite probable that there is a different molecular species hidden in band 3 which is associated with the anion transport in the neonatal red cell membranes. There is also a possibility that there is a specific interaction between the anion transport protein and the different microscopic environment provided in the neonatal red cell membranes presumably due to the different lipid composition [31,32], the different state of the spectrin complex [33], etc. in the vicinity of the transport protein. The lipid-protein interaction may alter the conformation of the transport system to lower the rate of transport [34,35] and also lower the energy barrier for the transport.

Note added in proof: (Received January 7th, 1982)

The apparent permeability P and the rate constant k would be 30% higher than the values reported here if intracellular pH of the cell suspensions calculated from data reported by Funder and Wieth [36] were used instead of our measured values. Our overestimation of the intracellular pH was probably caused by a failure to prepare the cell lysate under strict anaerobic conditions. A 30% increase in the absolute values of all the P and k values for both adults and newborns has no effect on the conclusions drawn in this work.

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