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LITHIUM TRANSPORT PATHWAYS IN HUMAN, CHICKEN AND EEL ERYTHROCYTES

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SUMMARY: The contribution of four transport pathways to Li^+ influx in human, chicken and eel erythrocytes was studied. All pathways were measured simultaneously in each kind of blood sample to avoid possible temporal variations in transport parameters. We found that: 1) Li^+ influx via Na^+-K^+ pump increased 2-3 fold in the order human < eel < chicken; 2) by the countertransport exchange system lithium influx increases its variance between 15% and 35% when saline medium is replaced by choline Cl; 3) the anion exchange system (band 3 protein) shows very little interindividual variability on the lithium influx; 4) the lithium leak pathway, for human and eel red blood cells, is the major contribution,whereas it is negligible for chicken erythrocytes. It is concluded that a similar transport system exists in the red cell membranes of the three species which can transport lithium. However,the exchange system does not exhibit identical transport characteristics in the three species and shows a marked inter- and intra-species variability in maximum transport capacity and some differences in susceptibility towards the inhibitors.

INTRODUCTION: Li^+ normally occurs in the body at trace levels only, it has been found to be the most effective drug for treating manic-depressive illnes (1). Lithium is remarkably toxic and for this reason it cannot be administered to the patients for a long period of time because of the lesions which it causes to the gastroenteric tube, to the nervous system and the to kidneys. In fact in pharmacology lithium is dosed out in small quantities (< 2 mM). In chemical studies, it has been found that the concentration of Li^+ in red cell water during Li^+ administration is usually only about one-third of that in blood plasma (2). Inasmuch as the steady-state concentration ratio between red cells and plasma for a passively distributed cation would be ~ 1.2, this observation suggests the presence of an uphill extrusion mechanism for Li^+. Both the site of Li^+, its therapeutic effect and the fundamental defect in manic-depressive illnes have been postulated to

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involve membranes (3). Besides, studies on the mechanism of lithium transport in human red cells have established that several systems can mediate the transfer of this cation across the cell membrane. It has been found that Na^+-K^+ pump ouabain sensitive can also transfer lithium into the red cell and promote active Na efflux in the absence of a competitive effect from external potassium (4). The effect of bicarbonate on the passive lithium permeability in human red cells has been investigated (5),(6).

These authors proposed that the increased permeability is due to the ability of carbonate to form ion pairs with sodium and lithium. According to this hypothesis, negatively charged ion pairs, $NaCO_3^-$ and $LiCO_3^-$, are transported through the cation-tight membrane by the specific anion exchange system, the band 3 protein. An ouabain-resitent Na^+-Li^+ countertransport system has been characterized (7) which can mediate Li^+ transport in both directions across the membrane of human erythrocytes. This countertransport is passive in nature and indipendent of energy supply by ATP. Furthermore the exchange of Na^+ for H^+ was first proposed as a mechanism for renal acidification many years ago. The operational properties of the Na^+/H^+ exchanger antiporter have been described in detail in more recent studies (8). In addition to Na^+ and H^+ the exchanger can transport . This system can be inhibited competitively by amiloride. Finally the leak pathways (9) found that a significant lithium influx arises from a leak that represents passive permeation, probably through pores in the membrane.

The present paper deals with the transport mechanisms of lithium across the red blood cell membrane and attemps to determine quantitatively and qualitatively the importance of each pathway. In addition to human erythrocytes, red cells of chicken and eel are studied. Avian and eel erythrocytes have somewhat fewer deletions in morphology and metabolic function capacity than the mammalian cell. For example: avian erythrocytes are post-mitotic cells which have sacrificed important functions to the cause of oxygen transport. Nevertheless, they still contain a nucleus, a few mitochondria, they synthesize protein and RNA (but not DNA), and depend on oxygen for their metabolic energy. They are permeable to water and small anions while being relatively impermeable to cations (10). However, the major functional difference is the inability to bind glyceraldehyde-3-phosphate dehydrogenase, a function associated with the NH_2 terminus of human band 3 (11). Even fish red blood cells contain a nucleus, they have been chosen because the kinetics of anion transport differ from those of mammalian red blood cells. In fact, in addition to a DIDS-sensitive (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid) component of anion transport there exists a considerable DIDS-insensitive component. Moreover, the temperature dependence of anion transport is about 15 Kcal/mol, instead of 32 as in human red blood cells. (12). With this demonstration of altered kinetic parameters in avian and in fish erythrocytes, differences in membrane structure can be expected at the specific sites involved in ion exchange and possibly other sites which may give rise to an altered disposition of the protein in the membrane. Furthermore, while literature on lithium transport in human erythrocytes is rapidly expanding and has been reviewed repeatedly, very little is known about the mechanism and control by which Li^+ crosses avian and fish red blood cell membrane.

MATERIALS AND METHODS

Preparation of the red blood cells

The studies were performed on fresh blood drawn from the antecubital vein of an apparently healthy human. The eel blood was drawn from the caudal vein using epharinized syringe after a soft anesthesia with ethyl-3 aminobenzoate-metanesulphonate (Sandoz MS-222) from subjects adjusted to fresh-water. The chicken blood was drawn from the jugular vein after having killed the animal. All the three types of blood were washed and centrifuged three times with an isotonic solution of NaCl. After this first phase, a hematocrit of 5% was done with the experimental solution "A" containing in mM: 130 NaCl, 20 TRIS, 10 glucose. In the experiments in which $NaHCO_3$ was required, it was added in the solution in replacement of glucose, and the solution was gassed for all the length of the experiment with 5% CO_2. Direct measurement confirmed that the pH did not change more than 7.4 ± 0.05. During the influx experiments the tubes were capped. For determinations of Li^+ uptake at extracellular Li^+ concentrations up to 10 mM, the cells were preincubated for 20-30 min. at the desired pH value (generally pH 7.4) before the Li^+ influx was initiated by adding aliquots of isotonic LiCl stock solutions. In experiments performed to study the action of inhibitors, these were added to the media either simultaneously with the red cells, or during the preincubation period 15 min prior to initation of Li^+ uptake. After the time intervals indicated in Results aliquots of the suspensions were rapidly cooled in an ice bath and the cells were washed three times in a tenfold excess of ice-cold isotonic lithium-free solution. Finally the cells were packed by 5 min centrifugation. After this phase, the red blood cells were hemolyzed in 4 ml distilled water and after centrifugation 3 ml of supernatants were drawn to measure lithium content by atomic absorption with Varian AA 1475 spectrophotometer. All cellular Li^+ concentration given in Results were expressed as mM x l. cells x h, by measuring hemoglobin as cyanomethemoglobin and converting to liter red blood cells. Measurements are quantified as mean values ± S.D. of individual experiments. The significance of observations was valuated by the student's T-test. Red cells were ATP-depleted by incubation for 2 h at 37°C, pH 7.4 in the presence of 10 mM inosine and 2 mM iodoacetate as described by the same authors (10). Similar results were obtained when ATP was depleted by incubating cells without substrate for as long as 36 h.

RESULT

Linearity of flux in relation to the Li^+ concentration

In order to determine the minimum concentration at which the flux remains linear, we have carried out some experiments, whose results as regards human, eel and chicken erythrocytes are shown in Fig. 1. As we can see, the influx increases linearly with $[Li^+]_o$ up to at least 10 mM, for all three kinds of erythrocytes.

CONTRIBUTION OF THE FOUR BASIC MECHANISMS TO LITHIUM MOVEMENT

To characterize the importance of Na^+-K^+ pump relatively to the lithium influx through the erythrocyte's membrane, Li^+ was measured in the saline medium "A" in absence (control) and in presence of 0.1 mM ouabain. The results are shown in Fig. 2. From it we can say that in all three kinds of cells is present a transport system exchanging Li^+ for Na^+. The activity of the Na^+-Li^+ exchange system varied up to 2 and 3 fold among individual red cell specimens from eel and chicken, the variability being much smaller in human erythrocytes. 50% of the Na^+-Li^+ exchange was blocked by 0.1 mM ouabain in chicken erythrocytes. This effect is less evident in the other two kinds of cells.

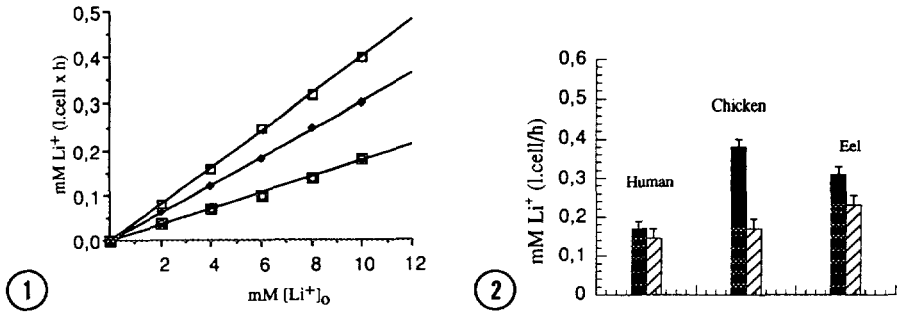

FIG. 1 - *Li^+ influx in human ◘ , eel ◆ and chicken □ red blood cells as a function of external Li^+ concentration. Cells from a single donor were incubated in NaCl saline with isotonic partial replacement of NaCl by LiCl. The experimental conditions are 37°C, pH 7.4. Abscissa represents cells Li^+ in unit of millimoles per liter red blood cell and per hour.*

FIG. 2 - *Li^+ influx (control ▮) and effect of ouabain ▨ in human, chicken and eel red blood cells. The concentration of ouabain was 10^{-4} M. Samples were taken after 1 h incubation, and intracellular Li concentration was determined after washing the cells in a cold $MgCl_2$ solution. For control and experimental measurements n= 6.*

In fact, in human and eel red blood cell the Na^+-K^+ pump contributes to about 15% of the lithium transport, in chicken red blood cell to 55%.

Na^+-Li^+ COUNTERTRANSPORT AND "LEAK"

The countertransport phenomenon is responsible for maintaining the small inner lithium concentration (13). From a phenomenological point of view, countertransport can be mediated by "porter" which can cross the membrane only if it has respectively linked Na^+ or Li^+ from each side of the membrane and can go back again to the starting point only if similarly charged. To put in evidence the countertransport contribution in lithium influx we used two techniques that abolish countertransport, we replaced NaCl with choline chloride, and we added ouabain.

From fig. 3 we can see that Li^+ influx increases in all three kinds of erythrocytes when NaCl is substituted with Choline Cl. This indicates that Li^+ enter the cell via the countertransport mechanism, presumably in exchange of Na_i^+. The flux increases between 18-35% and follows this order: chicken < eel < human. The "leak" transport of Li^+ occurs when Na ions are absent from both sides of the membrane and in the medium is present ouabain. If we compare column 2 and 3 we can say that in human red blood cells 50% of lithium influx cross the membrane by "leak", whereas in eel red blood cells this percentage is around 41%, and in chicken 15%.

THE BICARBONATE SENSITIVE PATHWAY

It has been shown previously (4) that bicarbonate stimulates influx of monovalent cations, including Li^+ (5) (6). This Li^+ movement is presumably related to the anion exchange system of the erythrocyty, i.e. the band 3 protein. To characterize lithium influx through the way sensible to $NaHCO_3$, a series of experiments has been programmed. The results are displayed on Fig. 4.

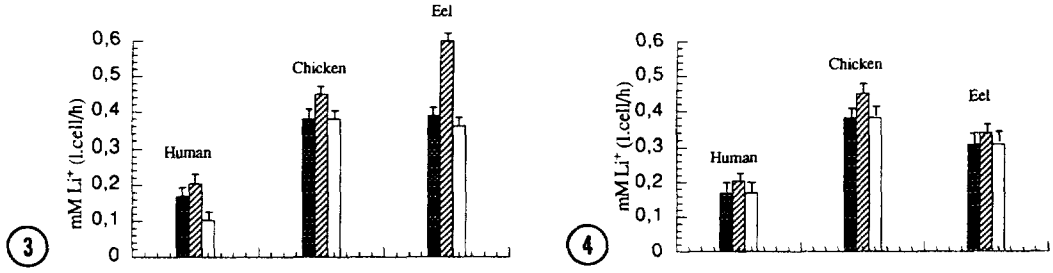

FIG. 3 - *Characterization of the Na^+ - Li^+ countertransport and "Leak" fractions of Li^+ influx. Choline Cl isotonically replaced NaCl. Temperature 37°C, pH 7.4. The medium of control was in mM: 130 NaCl, 20 TRIS, 10 LiCl, ± 0.1 mM ouabain. Abscissa is expressed as mM Li^+ (l.cell/h). NaCl ■ was substituted by Choline Cl in the experiments rapresented in columns 2 and 3*

FIG. 4 - *Characterization of the bicarbonate-sensitive fraction of Li^+ influx measured in saline medium in absence ■ and in presence of $NaHCO_3$ ▨ . The white column represents the Li^+ influx measured in presence of $NaHCO_3$ and 50 μM DIDS.*

In these experiments, the influence of Li^+ has been measured in saline medium "A" without $NaHCO_3$ (column 1) in presence of 10 mM $NaHCO_3$ (column 2) and in presence of $NaHCO_3$ + 50 μM DIDS (column 3). The results obtained allow us to state that the presence of $NaHCO_3$ determines the increase of lithium influence through the plasmatic membrane in the three types of red blood cells used. The percentage varies from 15 to 35% according to the kind of blood taken into consideration and this increase is inhibited by the presence of DIDS, since it is a specific inhibitor of the anion carrier and the addition of 50 μM of it causes the inhibition of lithium influx.This demonstrates that lithium follows the band 3 protein pathway.

CONCLUSION

One purpose of this work has been to investigate and to establish the contribution of the different mechanisms of lithium influx in human, chicken and eel red blood cells. The four main mechanisms investigated (Na^+-K^+ pump, countertransport Na^+-Li^+, bicarbonate-sensitive pathway, "leak"), contribute to lithium movement in the three kinds of blood used. The ouabain-sensitive Li^+ transport in all three kinds of cells is probably mediated by the Na^+-K^+ pump. If the external solution contains Na, Li influx into chicken red cell through this mechanism reaches a value of 0.4 mmol/(l. of cells x hour). In eel red blood cells it is 0.3 mmol/(l. of cells x hour) and 0.17 mmol/(l. of cells x hour) in human red blood cells (Fig. 2). Then we can say that the Na^+-K^+ pump contributes in minor measure in human and eel red blood cell, while its effect is more accentuated in chicken erythrocytes. The countertransport contributes to lithium transport in the different kinds of erythrocytes taken into consideration. A large capacity for sodium-lithium countertransport has been found in eel red blood cells, in contrast to the much smaller capacity of human and chicken red cells (Fig. 3). The molecular basis for these interindividual and species differences in countertransport is unknown. Bicarbonate causes selective increases of passive

lithium. The way sensible to bicarbonate is responsible for a moderate quantity of influx (Fig. 4). Funder et al. (1) calculated the contribution of the bicarbonate-stimulated influx in human red blood cell to be 66% of the total influx. This calculated contribution is much greater than the 9-17% we measure in eel < chicken < human. This difference can be attributed to the use of high Li^+ and $HCO3^-$ concentration in the experiments of Funder instead of the lower concentrations used in this paper. A possible explanation of the way followed by lithium in presence of NaHCO3 is that in this corse lithium (in presence of NaHCO3) may form $LiCO3^-$ ion pairs and only in this form, by its negative charge, it may be catalizated by the red cell anion exchanger. The remaining influx follows the way named "leak", it represents a passive permeation, probably through pores in the membrane. The experiments reported here demonstrate that the pathways for Li^+ influx into human, eel and chicken red cells share different features. A number of factors need to be taken into account when assessing their physiological relevance, ie.: differences in cellular Na^+ and K^+ content may contribute to the species variability but it is not sufficient to explain the differences discovered in the same mechanism in all three kinds of red blood cells. In the present paper we demonstrate that the four separable pathways are present for Li transport in human, chicken and eel red cells, but the mode of operation of these mechanisms differ in each kind of cells. Perhaps this could be because each species has to adapt the mechanism to its own living conditions.

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