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Cation transport hemolytic anemia
erythrocyte hereditary stomatocytosis

Cation Transport and Its Altered Regulation in Human Stomatocytic Erythrocytes

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Extract

Cation transport in a population of stomatocytic red blood cells (RBC) is abnormal in the following respects. First, active transport against a gradient, defined as the nonisotopic net accumulation of Na^+ or loss of K^+ induced by 0.1 mM ouabain, is markedly elevated (7.3 and 6.3 mEq/liter cells/hr for Na^+ and K^+, respectively), but the Na^+:K^+ active transport ratio is normal. Apparent uncoupling of the Na^+ and K^+ isotope transport is due to disproportionately increased ouabain-sensitive $^{24}Na^+$-$^{23}Na^+$ exchange (32.7 mEq/liter cells/hr), which is measured as a portion of $^{24}Na^+$ isotope efflux. Second, cation transport is unresponsive to variations in internal Na^+ concentration but decreases with decreasing extracellular Na^+.

Speculation

In the family we have studied, the clinical disorder, hereditary stomatocytosis, has been shown to be an autosomal dominant trait. The disorder may be due, therefore, to the transcription of an abnormal membrane protein which leads to altered shape, abnormal cation permeability, and reduced cell survival *in vivo*. The abnormal protein may be structural, leading to the shape alteration, and functional, leading to the alteration in permeability and transport. The abnormality, hereditary stomatocytosis, is a heterogeneous group of disorders; hence, the observations in this family may not reflect the specific membrane protein defect in all cases.

Hereditary stomatocytosis is a dominant pleomorphic disorder which often results in hemolytic anemia (10). Some stomatocytic RBC have been characterized by abnormalities in monovalent cation content and transport (10, 12). The stomatocytic RBC in two such families demonstrated a marked increase in Na^+ and K^+ transport with apparent uncoupling of the normal 3:2 Na^+:K^+ transport ratio (10, 12). RBC from three affected patients studied by Miller *et al.* (10) contained a moderately increased intracellular Na^+ (20.9 mEq/liter cells), slightly decreased intracellular K^+ (86.4 mEq/liter cells), and normal intracellular Na^+ plus K^+ content (107 mEq/liter cells). In one of these patients, Na^+ efflux was elevated, K^+ influx was normal, and the Na^+:K^+ transport ratio was strikingly abnormal (26:1). We have had the opportunity to study further the Na^+ and K^+ transport characteristics of the RBC from another affected member of this family.

The data indicate that in these particular stomatocytes, cation transport is abnormal in the following respects. First, active transport against a gradient, defined as the nonisotopic net accumulation of Na^+ or loss of K^+ induced by 0.1 mM ouabain, is markedly elevated, but the Na^+:K^+ active transport ratio is normal. Apparent uncoupling of Na^+ and K^+ isotope transport is due to disproportionately increased $^{24}Na^+$-$^{23}Na^+$ exchange measured as a portion of $^{24}Na^+$ isotope efflux. Second, cation transport is unresponsive to variations in internal Na^+ concentration but decreases with decreasing extracellular Na^+. This, of course, does not occur in normal RBC where transport is responsive to variation in internal Na^+ but unaffected by changing the external Na^+ concentration.

METHODS

RBC PREPARATION

Blood samples from *patient JM* and normal volunteers were obtained by venipuncture and anticoagulated with preservative-free sodium heparin (0.1 mg/ml). The cells were sedimented at 1,500 g and the plasma and buffy coat were removed. The RBC were then washed twice and resuspended to a packed cell volume of 40% in Krebs-Henseleit bicarbonate (KHB) buffer (sodium 145 mEq/liter and potassium 5.2 mEq/liter), pH 7.4, containing 10 mM glucose and 1 g/100 ml bovine serum albumin. The RBC were incubated in air plus 5% Co_2 as described previously (2).

ISOTOPE AND NET (ACTIVE) TRANSPORT AND EXCHANGE

Measurements of $^{24}Na^+$ exodus and $^{42}K^+$ accumulation were performed and calculated as reported previously (2). Total $^{24}Na^+$ transport represents the exodus of $^{24}Na^+$ from prelabeled RBC into the extracellular medium (*pathways 1 + 2 + 3* in Fig. 1). Ouabain-sensitive isotope transport is that proportion of the total which is inhibitable by 0.1 mM ouabain (*pathways 1 + 2*). This measurement has two components: active transport (*pathway 1*) and ouabain-sensitive exchange (*pathway 2*). Thus, ouabain-sensitive ^{24}Na efflux is not a measure of active sodium transport. Active cation transport (*pathway 1*) is a nonisotopic measurement and is determined by the intracellular gain of Na^+ (*pathway 4*) when *pathway 1* is inhibited by 0.1 mM ouabain. Ouabain-sensitive exchange (*pathway 2*) is calculated as the difference between ouabain-sensitive isotope transport and active transport. The residual isotope transport in the presence of 0.1 mM ouabain represents ouabain-independent exchange (*pathway 3*). For simplicity, only Na^+ movement is shown in Figure 1. K^+ behaves similarly in the opposite direction.

ACTIVE TRANSPORT AT VARIOUS INTRACELLULAR Na^+ CONCENTRATIONS

Elevation of the intracellular Na^+ content was achieved by incubating the RBC at 37° in KHB containing 10 mM glucose, 200 mg/100 ml albumin, and amphotericin B (obtained from Squibb as Fungizone) at a concentration of 5 μg/ml as described by Blum *et al.* (1). Increasing the time from 30 min to 4 hr of such incubations allowed the accumulation of additional intracellular Na^+. The amphotericin B was removed by washing twice with KHB containing glucose and 1 g/100 ml albumin followed by washing and resuspension in KHB containing glucose and 200 mg/100 ml

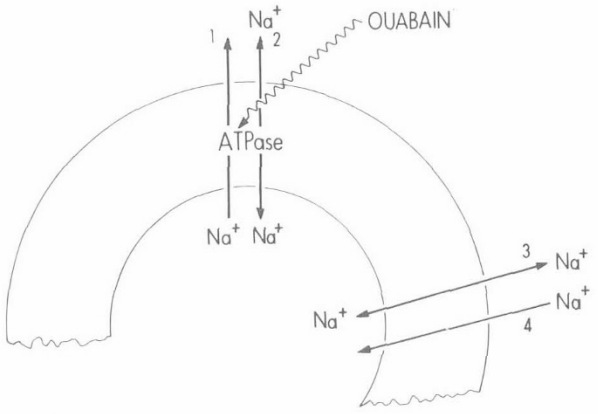

Fig. 1. Schematic representation of Na^+ movements across the red blood cell (RBC) membrane. Total $^{24}Na^+$ transport measures the total $^{24}Na^+$ passing from prelabeled RBC into the extracellular medium along *pathways 1 + 2 + 3*. Ouabain-sensitive $^{24}Na^+$ transport (*pathways 1 + 2*) is that portion of the total which is inhibitable by 0.1 mM ouabain and includes two components: active transport (*pathway 1*) and ouabain-sensitive exchange (*pathway 2*). Active transport (*pathway 1*) is a nonisotopic measurement and is determined as the intracellular accumulation of $^{23}Na^+$ (*pathway 4*) when *pathway 1* is inhibited by 0.1 mM ouabain. Ouabain-sensitive exchange (*pathway 2*) is calculated as the difference between ouabain-sensitive $^{24}Na^+$ transport (*pathways 1 + 2*) and active transport (*pathway 1*). The difference between total $^{24}Na^+$ transport (*pathways 1 + 2 + 3*) and ouabain-sensitive $^{24}Na^+$ transport (*pathways 1 + 2*) represents ouabain-independent exchange (*pathway 3*).

albumin (16). To decrease the intracellular sodium content of stomatocytic RBC, the cells were incubated in a modified KHB containing high K^+ (140 mEq/liter) and low Na^+ (5 mEq/liter) until the desired intracellular Na^+ was obtained. These cells were quickly washed three times with a standard KHB, resuspended, and incubated for measurement of active transport.

ACTIVE TRANSPORT AT VARIOUS EXTRACELLULAR Na^+ CONCENTRATIONS

Variation in the Na^+ gradient across the RBC membrane was produced by lowering the extracellular Na^+ concentration. Modified KHB containing 10 mM glucose and 1 g/100 ml albumin was prepared in which the Na^+ concentration was 15, 25, 60, 100, and 145 mEq/liter buffer. The diminished NaCl in each buffer was replaced by equiosmolar succinylcholine chloride which neither penetrated RBC nor affected transport. Normal and stomatocytic RBC were washed three times in the appropriate buffer and resuspended at a packed cell volume of approximately 40%. The RBC suspensions were then incubated at 37° in the presence and absence of ouabain, and timed samples were taken to determine active cation transport at each extracellular Na^+ concentration.

LACTATE PRODUCTION

Samples for lactate determination were removed periodically from the incubating cell suspensions and deproteinized by adding to 2 volumes 6% perchloric acid at 4°. The supernatant solution was removed and neutralized with 5 M K_2CO_3 and frozen for later analysis of lactate (9). Ouabain-sensitive lactate production was calculated from measurements of lactate production in the presence and absence of 0.1 mM ouabain.

STATISTICAL ANALYSIS

Slopes of regression lines were determined by the method of least squares, and means and standard deviations were performed by standard statistical methods (7).

RESULTS

ISOTOPE AND ACTIVE TRANSPORT AND EXCHANGE

Abnormalities in Na^+ and K^+ transport and exchange in these stomatocytes are shown in Table 1. Total $^{24}Na^+$ transport is more than 20 times normal, 60 mEq/liter cells/hr, compared with 2.9 mEq/liter cells/hr. Total $^{42}K^+$ transport is only 4–5 times normal, 7.5 mEq/liter cells/hr, compared with 1.7 mEq/liter cells/hr. Total isotope transport includes two components: ouabain-sensitive isotope transport and ouabain-independent exchange. Ouabain-sensitive $^{24}Na^+$ transport is also 20 times normal, 40.0 mEq/liter cells/hr, compared with 2.0 mEq/liter cells/hr. Ouabain-sensitive $^{42}K^+$ transport is 5 times normal, 6.5 mEq/liter cells/hr, compared with 1.3 mEq/liter cells/hr. The ouabain-independent exchange follows a pattern similar to the total isotope transport and ouabain-sensitive isotope transport. Ouabain-independent exchange of $^{24}Na^+$-$^{23}Na^+$ is 20.0 mEq/liter cells/hr, compared with normal, 0.9; ouabain-independent exchange of $^{42}K^+$-$^{39}K^+$ is 1.0 mEq/liter cells/hr, compared with 0.4.

In order to determine active (net) transport against a gradient, the rate of ouabain-induced gain of Na^+ and loss of K^+ were measured. Using this technique, the active transport of either Na^+ (7.3 mEq/liter cells/hr) or K^+ (6.3 mEq/liter cells/hr) is approximately 5 times normal. Consequently, the ratio of Na^+:K^+ transport is 1.2, which is identical with the ratio we obtained in normal cells. Ouabain-sensitive $^{24}Na^+$-$^{23}Na^+$ exchange is the difference between ouabain-sensitive $^{24}Na^+$ transport and active Na^+ transport. This exchange is markedly elevated, 32.7 mEq/liter cells/hr, compared with normal, 0.6. The magnitude of ouabain-sensitive $^{24}Na^+$-$^{23}Na^+$ exchange accounts for the disproportionate elevation of the ouabain-sensitive $^{24}Na^+$ exodus, since ouabain-sensitive $^{42}K^+$-$^{39}K^+$ exchange is near normal, 0.2 mEq/liter cells/hr, as compared with normal, 0.1 mEq/liter cells/hr.

ACTIVE TRANSPORT AT VARIOUS INTRACELLULAR Na^+ CONCENTRATIONS

Figure 2*A* shows the response of active Na^+ transport to variation of intracellular Na^+ concentration in normal and stomatocytic RBC. Active Na^+ transport was measured at intracellular Na^+ concentrations of 6–45 mEq/liter cells. While normal RBC increase transport to a maximum of 5 mEq/liter cells/hr at an intracellular Na^+ of approximately 30 mEq/liter cells, the stomatocytes have a greater maximal transport rate (7–8 mEq/liter cells/hr) which does not change with alteration of the intracellular Na^+. Figure 2*B* demonstrates a similar response of active K^+ transport to varying intracellular Na^+ concentration. Active K^+ transport, 6–7 mEq/liter cells/hr, is greater than the normal maximal K^+ transport 3.8 mEq/liter cells/hr, and is little affected by alteration of the internal Na^+ concentration. Values for active transport below an intracellular Na^+ of 6 mEq/liter cells could not be determined in the stomatocytes because the low intracellular Na^+ concentrations could not be maintained.

ACTIVE TRANSPORT VS. EXTRACELLULAR Na^+ CONCENTRATION

The effect of decreasing the extracellular Na^+ concentration on active Na^+ and K^+ transport is shown in Figure 3, *A* and *B*, respectively. In the stomatocytic RBC, both active Na^+ and K^+ transport were diminished from 9.6 and 7.7 mEq/liter cells/hr to 4.2 and 3.3 mEq/liter cells/hr, respectively, by lowering the extracellular Na^+ concentration from 140 mEq/liter to 20 mEq/liter. However, active Na^+ and K^+ transport in normal RBC was virtually unaffected by a similar reduction in the extracellular Na^+ concentration.

LACTATE PRODUCTION

Ouabain-sensitive lactate production is a measure of the metabolic energy devoted to active transport (2, 18). Figure 4 demon-

Table 1. *Na^+ and K^+ movements in normal and stomatocytic red blood cells (RBC)*[1]

	Normal RBC		Stomatocytic RBC	
	Na^+	K^+	Na^+	K^+
Total isotope transport	2.9 ± 0.3 (6)	1.7 ± 0.3 (6)	60.0	7.5
Ouabain-independent exchange	0.9 ± 0.2 (6)	0.4 ± 0.1 (6)	20.0	1.0
Ouabain-sensitive isotope transport	2.0 ± 0.2 (6)	1.3 ± 0.3 (6)	40.0	6.5
Active transport	1.4 ± 0.1 (7)	1.2 ± 0.1 (7)	7.3 ± 0.4 (7)	6.3 ± 0.9 (7)
Ouabain-sensitive exchange	0.6	0.1	32.7	0.2

[1] Values represent the mean ± SD in milliequivalents per liter cells per hr. The number of measurements is shown in parentheses.

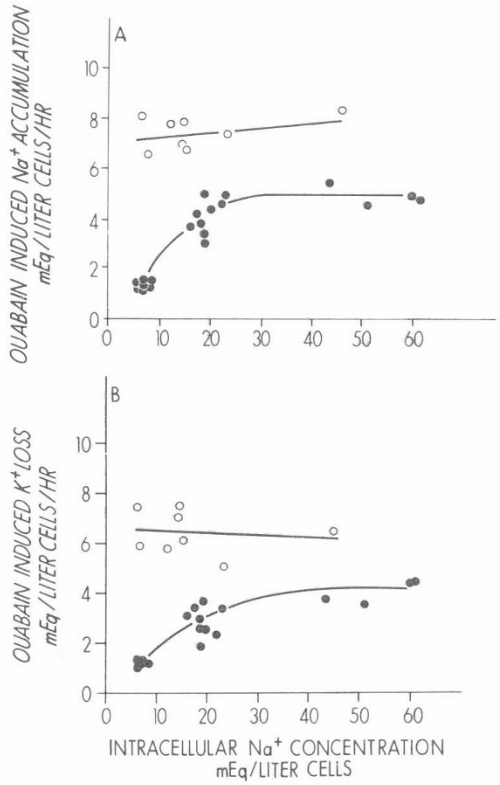

Fig. 2. The effect of various intracellular Na^+ concentrations on active transport, that is, the net intracellular accumulation of Na^+ (*A*), and loss of K^+ (*B*), induced by 0.1 mM ouabain. Each point represents the mean of duplicate measurements. ●, normal erythrocytes; ○, stomatocytes.

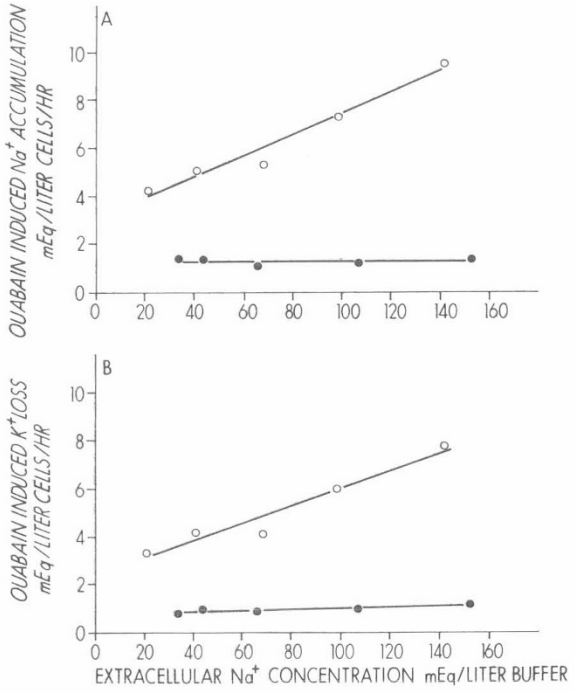

Fig. 3. The effect of various extracellular Na^+ concentrations on active transport, that is, the net intracellular accumulation of Na^+ (*A*), and loss of K^+ (*B*), induced by 0.1 mM ouabain. Slopes of the regression lines were determined by the method of least squares. The intracellular Na^+ concentration in stomatocytes ranged from 10.8 to 13.5 mEq/liter cells. ●, normal erythrocytes; ○, stomatocytes.

strates the relationship between active Na^+ transport and the corresponding energy requirement. In normal RBC, the lactate production required for the transport of 3 mEq Na^+/liter cells/hr is approximately 1 mM/liter cells/hr. The markedly elevated active transport rate in these stomatocytes permits graphic extension of the normal relationship between transport and metabolism.

DISCUSSION

Na^+ and K^+ transport in the human erythrocyte is responsible for the maintenance of a low intracellular Na^+ concentration and a high intracellular K^+ concentration. This transport is mediated by an ATPase system located in the erythrocyte membrane (15, 17). Na^+ and K^+ transport opposes the respective cation gradients, and usually occurs in a ratio of approximately 3:2 (Na^+:K^+) (6, 8, 13). The dissociation of Na^+ and K^+ transport has never been satisfactorily documented in human erythrocytes. Pump dissociation secondary to substrate depletion (2) was subsequently shown to be an artifact of increasing ouabain-sensitive K^+-K^+ exchange in depleted RBC (14, 16). The ratio of Na^+ to K^+ transport in the

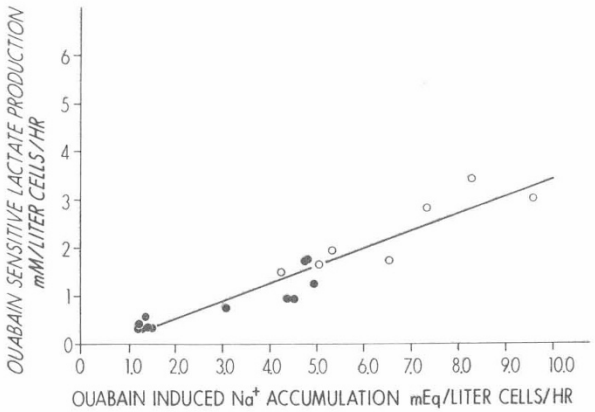

Fig. 4. The relationship between ouabain-sensitive lactate production and active Na^+ transport, that is, the net intracellular accumulation of Na^+ induced by 0.1 mM ouabain. Each point represents the mean of duplicate measurements. The line was fitted to the points by the method of least squares. ●, normal erythrocytes; ○, stomatocytes.

RBC of several families with stomatocytosis has also been dissociated (10, 12). In this study, we have had the opportunity to evaluate more extensively both the isotopic and net movements of Na^+ and K^+ in the RBC of one such patient. Here, the disparity in Na^+ to K^+ transport, as measured by isotopes, can be explained by a large component of nonactive $^{24}Na^+$-$^{23}Na^+$ exchange. When active transport is defined as the net accumulation of intracellular Na^+ and loss of intracellular K^+ induced by 0.1 mM ouabain, the transport ratio is, in fact, normal.

However, there is marked elevation of both Na^+ and K^+ transport in these stomatocytes. Na^+ transport is persistently greater than 6.5 mEq/liter cells/hr, and K^+ transport is greater than 5.5 mEq/liter cells/hr, compared with a normal maximal transport of Na^+ 5.0 mEq/liter cells/hr and K^+ 3.8 mEq/liter cells/hr. These normal maximal values agree well with those reported by Garay and Garrahan (3). However, the cell population in this patient with stomatocytosis contains 15–25% reticulocytes, and shows a marked elevation of the Na^+-K^+-ATPase (10). We pose this as a potential explanation for the high transport rate seen in this patient's RBC.

The control of transport, however, is less easily explained. These stomatocytes are markedly permeable to both Na^+ and K^+ and demonstrate a striking abnormal persistence of maximal transport throughout a range of intracellular Na^+ between 6 and 45 mEq/liter cells. A decrease in transport could only be achieved by reducing the gradient across the cell membrane. This maneuver ordinarily has little or no effect on cation transport (4), and Na^+ and K^+ transport is normally responsive only to variation of the intracellular Na^+ concentration (3). Our data in normal cells are comparable with those published by Garrahan and Glynn (4) and Garay and Garrahan (3). Reticulocyte-rich blood cells which do not have a permeability defect have normal net transport rates and a normal response to elevation of the internal Na^+ (16). It is likely that passive cation movement across the plasma membrane in the direction of the cation gradient is so great in these stomatocytes that the transport mechanism responds to this, rather than the actual concentration of Na^+ within the cell. We could not test whether very low intracellular Na^+ concentration influenced transport.

The ATP hydrolysis devoted to active cation transport is reflected in the ouabain-sensitive lactate production since approximately 1 mol ATP is generated for each mole of lactate produced if one assumes little metabolite passage through the Rapoport-Luebering shunt. Three milliequivalents Na^+ and 2 mEq K^+ are transported against the respective gradients for each millimole of ATP hydrolyzed or ouabain-sensitive lactate produced (5, 17, 18). Transport-related lactate production in stomatocytes is consistent with this relationship at very high rates of transport, increasing the accuracy of the measurements. Varying the transport rate in these cells demonstrated further that the relationship between transport and transport-related lactate production remained linear. This continuous linear relationship which is independent of the cells' permeability abnormality provides further support that ouabain-sensitive net transport is an accurate measurement of true active transport.

SUMMARY

These studies define further the abnormalities of Na^+ and K^+ movements in stomatocytic RBC. We have found total and ouabain-sensitive transport of radiolabeled Na^+ and K^+ to be markedly greater than normal. The increased Na^+ transport relative to K^+ transport as measured by isotopes is due to an increased ouabain-independent and ouabain-sensitive $^{24}Na^+$-$^{23}Na^+$ exchange. $^{42}K^+$-$^{39}K^+$ exchanges remain relatively normal. Active Na^+ and K^+ transport is elevated but remains coupled in a normal ratio.

In contrast to normal RBC, active transport in these stomatocytes is not significantly influenced by variations in intracellular Na^+ concentration between 6 and 45 mEq/liter cells. However, reducing the gradient across the RBC membrane by decreasing the extracellular Na^+ results in a linear fall in active transport. These stomatocytes thus demonstrate an altered control mechanism for active cation transport.

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