Sodium transport in erythrocytes: differences between normal children and children with primary and secondary hypertension

M UCHIYAMA, V SHAH, C E DAMAN WILLEMS, AND M J DILLON

Renal Unit, Hospital for Sick Children, Great Ormond Street, and Department of Paediatric Nephrology, Institute of Child Health, London

SUMMARY The following measurements were made in normal children, children with primary hypertension, and children with secondary hypertension: erythrocyte intracellular sodium concentration, total sodium efflux rate constant, and maximum binding of ouabain to erythrocytes reflecting the number of sodium/potassium adenosine triphosphatase pump sites. Children with primary hypertension had a significantly higher mean erythrocyte intracellular sodium concentration (8.2 compared with 6.6 and 6.7 mmol/l cells), and significantly lower total sodium efflux rate constant (0.5071 compared with 0.6983 and 0.6197) and maximum binding of ouabain to erythrocytes (9.1 compared with 11.7 and 11.0 nmol/l cells) than normal children and children with secondary hypertension, respectively.

Since the original report by Losse *et al* in 1960¹ that intracellular sodium was increased in the red blood cells of patients with essential hypertension, various abnormalities of ion transport have been reported in patients with primary hypertension,^{2–4} and it has been postulated that essential hypertension may be caused by disturbance of membrane electrolyte transport. Because it is difficult to study the cells in the walls of blood vessels where abnormalities may affect blood pressure, circulating blood cells—for example, leucocytes and erythrocytes—have been used to study the transport of electrolytes across membranes.

Few studies have been undertaken on transport of sodium across cell membranes in children with hypertension.^{5–6} We therefore undertook a study of erythrocyte sodium transport in children with primary hypertension and compared the findings with those in normal children and in patients with secondary hypertension.

Subjects and methods

We studied 15 normal children who acted as controls (range 5–15 years), 12 children with primary hypertension (age range 12–16 years), and 27 children with secondary hypertension (age range 5–15 years). The control children, whose blood was being taken for another reason during the course of investigations of minor illnesses, did not have any family

history of hypertension. Children with primary hypertension showed significant increases in blood pressure (greater than the 95th centile for age) on repeated measurements and were not receiving anti-hypertensive treatment. No other causes for their hypertension were found on thorough examination. All patients with secondary hypertension had renal hypertension, and in most of these the blood pressure was renin dependent. All the children with primary hypertension had normal plasma renin activities for their ages (less than 899 ng angiotensin I/l/hour or 690 ng angiotensin I/l/hour). Eight of 27 children with secondary hypertension were receiving treatment; six were receiving propranolol and hydralazine, and two were receiving captopril. The study was approved by the ethical committee of the Hospital for Sick Children and the Institute of Child Health.

Samples of 5 ml of fresh heparinised blood were taken; 2 ml of each were used for measurement of the ²²sodium efflux, and the remaining 3 ml were separated into plasma and erythrocytes at 3000 rpm.

The erythrocyte intracellular sodium concentration was measured by flame photometry with IL 943 flame photometer (Instrumental Laboratory) after washing erythrocytes three times with 155 mmol/l of cold choline chloride (4°C). The ²²sodium efflux rate constant was measured by a modification of the method used by Fitzgibbon *et al*⁷; 4.0 µCi of ²²sodium (22 µCi/ml, Amersham International) was

added to 2 ml of whole blood and incubated at 37°C for one hour. Labelled erythrocytes were obtained and washed four times with cold isotonic saline. A sample of 250 µl of packed erythrocytes was added to 500 µl of their own plasma, and reincubated at 37°C for one hour; 25 µl of a cell suspension were taken into counting tubes both before and after this incubation. Finally, 250 µl of incubated blood was centrifuged at 12000 rpm for three minutes (Eppendorf microcentrifuge) to obtain 125 µl of supernatant for counting.

In each tube ^{22}Na was counted with a LKB gamma counter (1282 Compugamma). The efflux rate constant (K_e) was determined by using the following equation: $\ln(1 - N_t/N_0) = -K_e t$, where $N_0 = ^{22}\text{Na}$ sodium present/unit volume of suspension of incubating cells (mean of the counts/unit volume of suspension taken at time 0 and 60 minutes), $N_t = ^{22}\text{Na}$ sodium present per unit volume of supernatant at time 60 minutes.

The binding of ouabain to erythrocytes was measured by a modification of the method used by Gardner and Conlon.⁸ Packed erythrocytes, which were washed three times with cold isotonic saline (4°C), were resuspended in trometamol (TRIS) buffer (sodium chloride 130 mmol/l, sucrose 20 mmol/l, glucose 10 mmol/l, and TRIS 10 mmol/l, pH 7.4) to a packed cell volume of 0.15. A stock solution of ouabain (100 mmol/l in 10% ethanol) was prepared and kept at 4°C.

A sample of 100 µl of ^3H ouabain (37 Ci/ml, Amersham International) was placed into a glass tube and evaporated under nitrogen. The residue was redissolved with 500 µl of isotonic saline, of which 100 µl was diluted in 2.6 ml of isotonic saline on assay. Two fold serial dilutions were then made four times. Of the erythrocyte suspension, 300 µl were incubated with 50 µl of the diluted radioactive ouabain at each dilution, and with 50 µl of isotonic saline or 1 mmol/l of cold non-radioactive ouabain at 37°C for two hours. Subsequently the unbound ouabain was removed by washing three times with isotonic saline, and the final pellet resuspended in 200 µl of 10% trichloroacetic acid. After centrifuging 100 µl of its supernatant was used for liquid scintillation counting (LKB; 1210 Ultro-beta) in 3 ml of Cocktail T (British Drug Houses).

Non-specific binding was estimated by replacing 50 µl of isotonic saline with 50 µl of 1 mmol/l of cold ouabain. The amount of ouabain specifically bound was calculated by subtracting the non-specific counts from the total counts. Maximum binding of ouabain to erythrocytes was calculated using a curve fitting computer programme. All determinations were calculated in duplicate.

Intra-assay coefficients of variation were 2.4%,

3.6%, and 6.2% for intracellular sodium concentration, total sodium efflux rate constant, and maximum binding of ouabain to erythrocytes, respectively. Results were expressed as mean (SEM) and analysed parametrically by the unpaired Student's *t* test.

Results

In the children with primary hypertension, mean erythrocyte intracellular sodium concentration was significantly higher (8.2 (0.63) mmol/l cells) than in

Fig 1 Intracellular sodium concentration in erythrocytes from normal children, children with primary hypertension, and children with secondary hypertension.

Fig 2 Total sodium efflux rate constants in erythrocytes from normal children, children with primary hypertension, and children with secondary hypertension.

control children (6.6 (0.32) mmol/l cells), and in children with secondary hypertension (6.7 (0.26) mmol/l cells) ($p < 0.05$) (fig 1). In children with primary hypertension, mean total sodium efflux rate constant was significantly lower (0.5071 (0.04)) than in control children (0.6983 (0.04)), and in children with secondary hypertension (0.6197 (0.03)) ($p < 0.001$) (fig 2). The mean maximum binding of ouabain to erythrocytes that reflects the number of pump receptor sites was reduced in children with primary hypertension (9.1 (0.45) nmol/l cells) compared with controls (11.7 (0.73) nmol/l cells), and with those with secondary hypertension (11.0 (0.49) nmol/l cells) ($p < 0.005$) (fig 3).

Discussion

There are several pathways for active transport of

sodium in erythrocytes. Firstly, there is sodium/lithium countertransport, which is equivalent to sodium/sodium countertransport. This channel is thought to exchange a single external sodium ion for a single internal one. This cannot affect the overall distribution of sodium across cell membranes and there is, therefore, some doubt about its physiological role.⁹ Canessa *et al*,¹⁰ however, reported that countertransport was increased in erythrocytes in patients with essential hypertension, suggesting that this system could be a marker for essential hypertension.

The second system that has been studied is frusemide sensitive sodium/potassium cotransport. This pathway lies dormant in the cell membrane unless intracellular sodium concentrations rise above a critical point, and acts as a supporting mechanism for sodium extrusion. Garay *et al* reported that the activity of this system was decreased in erythrocytes of patients with essential hyper-

Fig 3 Binding of ouabain to erythrocytes representing pump receptor sites in normal children, children with primary hypertension, and children with secondary hypertension.

tension, suggesting that this system could also be a marker for essential hypertension.¹¹

A third system is the sodium pump, which is the best described and most widely studied pathway for cellular sodium homeostasis. The activity of the pump system is consistently low in white cells of adult patients with essential hypertension,^{12,13} but inconsistent results have been obtained in erythrocytes that are thought to be caused by the activity of the pump being much slower in erythrocytes than in white cells.⁹ It was impossible to study white cells in children, however, because more than 100 ml of blood would be required for each assay, so we chose to study erythrocytes by measuring erythrocyte intracellular sodium concentration, total sodium efflux rate constant and maximum binding of ouabain to erythrocytes instead of sodium/lithium counter-transport or sodium/potassium cotransport.

Few studies have been undertaken on erythrocyte sodium transport in children with primary hypertension. Mongeau^{5,6} reported that children with labile hypertension tended to have decreased sodium/potassium cotransport and those with stable hypertension increased countertransport. Although interesting, however, these findings may not contribute to our understanding of the mechanisms concerned in developing hypertension, because the methods used for measuring sodium transport either reflect activity of a system with little physiological importance or rely on laboratory induced unphysiological conditions.

In the present study eight children with secondary hypertension were receiving antihypertensive drugs (captopril, propranolol, or hydralazine). We did not find any differences in erythrocyte sodium transport between two groups of children receiving or not receiving hypotensive treatment, even though the drugs used have been reported to have some effect on sodium transport across the cell membranes in adults.¹⁴ The children who acted as controls were age matched with the children with secondary hypertension, but the children with primary hypertension were a little older. We have not, however, observed any differences in erythrocyte sodium transport associated with age in children after infancy. Differences in age and medication were therefore negligible among the three study groups.

Our findings that children with primary hypertension had significantly higher erythrocyte intracellular sodium concentration and significantly lower total sodium efflux rate constant and maximum binding of ouabain to erythrocytes than control children and children with secondary hypertension, confirm results reported by workers studying adults with hypertension. It seems likely that the high erythrocyte intracellular sodium

concentration seen in children with primary hypertension is a result of low total sodium efflux rate constant caused by the small number of sodium pump sites.

These findings suggest that measurements of erythrocyte intracellular sodium concentration, total sodium efflux rate constant, and maximum binding of ouabain to erythrocytes may have some diagnostic value in positively identifying children with primary as opposed to secondary hypertension, and may have a predictive value in identifying children who are at risk of developing essential hypertension in adult life. There is, however, substantial overlap among the groups of patients and further study is currently being undertaken to establish whether factors such as a family history of essential hypertension play a part in modifying transport of electrolytes across cell membranes. It is not yet known whether the erythrocyte sodium transport defect is caused by the hypertension, is a cause of the hypertension, or is just a marker.⁹

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Correspondence to Dr MJ Dillon, Renal Unit, Hospital for Sick Children, Great Ormond Street, London WC1 3JH.

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