Erythrocyte sodium transport in chronic renal failure

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(Received 3 August 1981; accepted 3 December 1981)

Summary

1. Erythrocyte sodium, sodium transport (ouabain-sensitive efflux rate of sodium, $\dot{M}^{\text{Na}}_N$, and ouabain-sensitive efflux rate constant of sodium, $K^{\text{Na}}_N$), sodium-potassium activated ouabain-sensitive adenosine triphosphatase (Na$^+$,K$^+$-ATPase) activity and [$^3$H]ouabain-binding capacity were measured in 15 patients with chronic renal failure and in 10 healthy subjects.

2. As a group, patients with chronic renal failure had a lower erythrocyte sodium and $\dot{M}^{\text{Na}}_N$ compared with healthy subjects.

3. When patients were divided according to their erythrocyte sodium (greater or less than 4 mmol/kg of cells), in the group of patients whose erythrocyte sodium was less than 4 mmol/kg of cells (group A) the $K^{\text{Na}}_N$ was higher than that in healthy subjects and the $\dot{M}^{\text{Na}}_N$, Na$^+$,K$^+$-ATPase activity and [$^3$H]ouabain-binding capacity were the same as those in healthy subjects. In the subgroup of patients with renal failure whose erythrocyte sodium content was greater than 4 mmol/kg of cells (group B) the $K^{\text{Na}}_N$ was less and plasma urea concentration higher than in group A and Na$^+$,K$^+$-ATPase activity, [$^3$H]ouabain-binding capacity and $\dot{M}^{\text{Na}}_N$ were lower than in healthy subjects.

4. These results suggest that in early chronic renal failure there is stimulation of ‘sodium pumps’ (without alteration in their number), which causes a lowering of erythrocyte sodium content, and that as the disease progresses there is inhibition of the ‘sodium pumps’ as well as a reduction in membrane permeability so that erythrocyte sodium is near normal.

Key words: Na$^+$,K$^+$-activated adenosine triphosphatase, chronic renal failure, erythrocytes, sodium transport.

Introduction

The sodium content of all cells including erythrocytes is severalfold less than that of plasma. This concentration gradient is maintained by active transport of sodium out of the cells. The enzyme responsible for this process is sodium-potassium-activated ouabain-sensitive adenosine triphosphatase (Na$^+$,K$^+$-ATPase, EC 3.6.1.3).

In chronic renal failure abnormalities in membrane function have been demonstrated. Welt et al. [11] reported that in some uraemic patients erythrocyte sodium content was increased and that this was coupled with a decrease in the rate constant for sodium efflux and a decrease in ouabain-sensitive ATPase activity in the erythrocyte membrane. Cole et al. [2] showed that this inhibition of sodium pump activity was caused by a ‘factor’ in the plasma. Later Cole [3] and Kramer et al. [4] confirmed the decrease in erythrocyte ATPase in chronic renal disease. A similar defect in sodium transport in leucocytes has been demonstrated in uraemia [5]. These observations suggest that the defect in sodium transport may be widespread in all tissues.

However, in a study of the relationship between erythrocyte sodium content and sodium transport [6], it was observed that in chronic renal failure erythrocyte sodium content was not raised. We therefore decided to investigate...
erythrocyte sodium transport in chronic renal failure in detail.

Methods

Subjects

In this study 15 patients attending a renal clinic were studied. These patients were sufficiently ill to attend the renal clinic regularly, but not ill enough to require dialysis. There were nine males and six females, whose age range was 20–57 years.

As a control group ten staff members of the laboratory, four females and six males whose age range was 23–42 years, were studied.

In patients and control subjects 20 ml of blood was taken from an antecubital vein and placed in lithium/heparin tubes. The following measurements were made on this blood sample: plasma electrolyte concentrations, erythrocyte sodium concentration, erythrocyte ouabain-sensitive efflux rate of sodium ($\delta M_{Na}$) and the ouabain-sensitive efflux rate constant of sodium ($\delta K_{Na}$), Na$^+$.K$^+$-ATPase activity and $[^3H]$ouabain-binding capacity.

The methods used to measure erythrocyte sodium content ([Na]$^{\text{RBC}}$), the ouabain-sensitive efflux rate of sodium ($\delta M_{Na}$) and the ouabain-sensitive efflux rate constant of sodium ($\delta K_{Na}$) have been previously described [7].

Erythrocyte sodium content

Heparinized blood was centrifuged at 1500 g for 10 min. The plasma and buffy coat were removed and samples of the packed cells were transferred from the bottom of the cell column into soft polythene Beckman centrifuge tubes (500 µl), which were centrifuged at 15,000 g for 30 min at 4°C in a Beckman Microfuge. Each centrifuge tube was cut 3 mm and 20 mm above its tip and the middle segment was then weighed and dropped into 10 ml of lithium sulphate solution (15 mmol/l), which was shaken until the segment was empty and the cells were haemolysed. The empty segment was dried and weighed and the weight of packed cells that had been haemolysed was calculated. The sodium and potassium concentrations of the haemolysates were measured by flame photometry. Two samples of packed cells were taken from each blood sample and the results given for erythrocyte sodium content are the means of these two values. The measurements of sodium and potassium content were made on either unwashed erythrocytes ([Na]$^{\text{RBC}}$) or erythrocytes washed three times with magnesium chloride solution (285 mosmol/kg) before centrifugation, to remove plasma ([Na]$^{\text{RBC}}$). Only the true or washed erythrocyte sodium content ([Na]$^{\text{RBC}}$) values are given in the Results section.

Na$^+$.K$^+$-ATPase activity

Packed cells (0.5 ml in a plastic tube) were immersed in methanol/solid carbon dioxide until they were frozen, and were thawed by immersion in a water bath at 37°C. The freezing and thawing was repeated three times, after which 0.5 ml of water was added to the cells. ATPase activity was measured as the rate of release of inorganic phosphate from ATP at pH 7.6 and 37°C under the assay conditions described by Huang & Askari [8]. A portion (0.5 ml) of each haemolysate was added to 2.0 ml of buffer solution to give the final concentrations (mmol/l) ATP 2, Na$^+$ 100, K$^+$ 25, Mg$^{2+}$ 3 and EDTA 1. The haemolysates were incubated in duplicate and to one of them was added 0.1 ml of ouabain solution ($10^{-2}$ mol/l). The mixtures were incubated at 37°C for 2 h and the reaction was stopped by the addition of 2.0 ml of trichloroacetic acid ($10^{-1}$ mol/l). The amount of phosphate in the haemolysate before incubation was measured by adding another sample of the haemolysate to the buffer and immediately adding the trichloroacetic acid. The phosphate concentration in the mixtures was measured on a Mark I Auto-Analyzer by the method of Lawrence [9]. The haemoglobin concentration of the haemolysates was measured after conversion into cyanomethaemoglobin [10]. The ATPase activity of the haemolysate was calculated as µmol of phosphate released h$^{-1}$ g$^{-1}$ of haemoglobin. Na$^+$.K$^+$-ATPase (ouabain-sensitive ATPase) activity was calculated as the difference between the ATPase activity in the absence and presence of ouabain.

Sodium transport [7]

The ouabain-sensitive efflux rate of sodium was measured indirectly in whole blood by incubating heparinized blood at 37°C in the presence of ouabain. Three 3 ml aliquots of heparinized blood were pipetted into 5 ml tubes. To one of these 20 µl of 20% ethanol was added and to others ouabain in 20% ethanol was added to give a final concentration of $10^{-7}$ mol/l. The tubes were incubated at 37°C for 2 h. At the end of 2 h the sodium content in erythrocytes in all the tubes was determined ([Na]$^{\text{RBC}}$). The ouabain-sensitive efflux rate of sodium ($\delta M_{Na}$) was calculated from the difference between
Sodium transport in renal failure

**TABLE 1. Erythrocyte sodium content, ouabain-sensitive sodium transport, Na+, K+-ATPase activity, [H]ouabain-binding capacity and the plasma concentrations of urea, creatinine and potassium in healthy subjects and patients with chronic renal failure**

Means ± SEM are shown. Significance of differences from healthy subjects:

***P < 0.001; **P < 0.02; *P < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 10)</th>
<th>Chronic renal failure (n = 15)</th>
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<tbody>
<tr>
<td>Erythrocyte sodium ([(\text{Na}^{+})(_{\text{sc}})] (mmol/kg of cells))</td>
<td>6.55 ± 0.28</td>
<td>4.87 ± 0.28***</td>
</tr>
<tr>
<td>Erythrocyte sodium ([(\text{Na}^{+})(_{\text{sc}})] (mmol/kg of cells))</td>
<td>88.0 ± 0.89</td>
<td>89.3 ± 0.72</td>
</tr>
<tr>
<td>Ouabain-sensitive efflux rate constant of sodium ([(\text{K}^{-})(_{\text{sc}})] (h(^{-1})) )</td>
<td>0.240 ± 0.013</td>
<td>0.247 ± 0.026</td>
</tr>
<tr>
<td>Ouabain-sensitive efflux rate of sodium ([(\text{M}\text{f}^{+})(_{\text{sc}})] (mmol h(^{-1}) kg(^{-1})) )</td>
<td>1.62 ± 0.078</td>
<td>1.22 ± 0.110**</td>
</tr>
<tr>
<td>Na+, K+-ATPase activity ([(\text{p}\text{mol} \text{ of} \text{P, h}^{-1}\text{ g}^{-1}\text{ of haemoglobin})]</td>
<td>6.61 ± 0.61</td>
<td>5.01 ± 0.49</td>
</tr>
<tr>
<td>[H]ouabain-binding capacity ([(\text{H}\text{f}^{-})(_{\text{sc}})] (c.p.m./10(^6) cells))</td>
<td>19.0 ± 0.68</td>
<td>15.8 ± 1.07*</td>
</tr>
<tr>
<td>Plasma urea (mmol/l)</td>
<td>3.8 ± 0.2</td>
<td>29.7 ± 2.2***</td>
</tr>
<tr>
<td>Plasma creatinine (mmol/l)</td>
<td>93 ± 4</td>
<td>843 ± 67***</td>
</tr>
<tr>
<td>Plasma potassium (mmol/l)</td>
<td>4.2 ± 0.10</td>
<td>4.7 ± 0.21</td>
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**Plasma electrolytes**

The concentrations of plasma sodium, potassium, bicarbonate, urea and creatinine were measured on a Vickers Multichannel Analyser M300.

The group data are given as means ± SEM and the groups were compared by using unpaired t-tests. A P value of 0.05 or less was considered significant.

**Results**

Table 1 shows the results of studies performed on both patients and healthy subjects. The plasma concentration of potassium was normal in the group with chronic renal failure and plasma concentrations of urea and creatinine were higher but varied widely. The erythrocyte sodium content in the patients with chronic renal failure was lower than that in the healthy subjects. The ouabain-sensitive efflux rate constant of sodium ([\(\text{K}^{-}\)\(_{\text{sc}}\)] and Na+,K+-ATPase activity were not different but the ouabain-sensitive efflux rate of sodium ([\(\text{M}\text{f}^{+}\)\(_{\text{sc}}\)] and [H]ouabain-binding capacity were lower in the patients with chronic renal failure.

To facilitate the analysis of the data further, the group of patients was divided arbitrarily into those whose erythrocyte sodium content was less than 4 mmol/kg of cells (group A) and those whose erythrocyte sodium content...
was greater than 4 mmol/kg of cells (group B) (the lower limit of the normal range being 4 mmol/kg of cells) and the results are shown in Table 2. The important observations are that the ouabain-sensitive efflux rate constant of sodium (\(\Delta V_{0}^{Na^{+}}\)) was higher in group A (patients whose erythrocyte sodium content was less than 4 mmol/kg of cells), but the ouabain-sensitive efflux rate of sodium (\(\Delta V_{0}^{Na^{+}}\)), \(Na^{+},K^{+}\)-ATPase activity and \([3H]\)ouabain-binding capacity were not different when compared with values in healthy subjects. In group B (patients whose erythrocyte sodium content was greater than 4 mmol/kg of cells) the ouabain-sensitive efflux rate constant of sodium (\(\Delta V_{0}^{Na^{+}}\)) was less and the plasma urea concentration was higher than the values in group A, and the ouabain-sensitive efflux rate of sodium (\(\Delta V_{0}^{Na^{+}}\)), \(Na^{+},K^{+}\)-ATPase activity and \([3H]\)ouabain-binding capacity were lower than in healthy subjects.

Discussion

Welt et al. [11] first demonstrated an abnormality in cation transport in chronic renal failure. Welt et al. [1] later showed that 25% of patients with chronic renal failure had a higher erythrocyte sodium content than normal and that this was associated with a decreased efflux rate constant and \(Na^{+},K^{+}\)-ATPase activity. In a more recent study Cole [3] reported decreased erythrocyte \(Na^{+},K^{+}\)-ATPase activity in 19 out of 20 patients with chronic renal failure and in three patients erythrocyte sodium content was very much higher than in control subjects. Kramer et al. [4] reported an increased erythrocyte sodium content in seven out of 13 patients and moderately decreased \(Na^{+},K^{+}\)-ATPase activity and a doubling of the Michaelis constant for ATP (\(K_{m}^{\text{ATP}}\)) in chronic renal failure. However, in the group of patients we studied we not only failed to find an increase, but the erythrocyte sodium concentration was found to be lower than in the control subjects. This could have been due to selection of patients with better renal function than those of Cole [3] and Kramer et al. [4]. However, there were no significant differences between the present and previous studies [3, 4] in plasma creatinine and urea concentrations, which were used as indices of renal function.

We have previously noted [6] the low erythrocyte sodium content in chronic renal failure, which has also been observed by others [12, 13]. We measured ouabain-sensitive efflux...
rate of sodium \((^{\text{d}}M^{\text{Na}}_{\text{Na}})\) and the ouabain-sensitive efflux rate constant of sodium \((^{\text{d}}K^{\text{Na}}_{\text{Na}})\) by methods which have been validated previously [7]. In the technique of measuring sodium transport, whole blood from the patient was used so that the transport activity measured was similar to that found in vivo, unlike other techniques in which the cells are washed and incubated in an artificial medium. In these latter techniques it could be argued that any inhibitor or activator originally present in whole blood might be removed. Thus the finding of a low erythrocyte sodium content in both washed and unwashed erythrocytes (not presented), in spite of the use of techniques which do not remove endogenous inhibitor, is highly significant.

In addition to measuring sodium transport we measured the Na\(^{+},K^{+}\)-ATPase activity and \([\text{H}]\text{ouabain-binding capacity}. The Na\(^{+},K^{+}\)-ATPase activity measured under optimal conditions could be considered to measure the number of enzyme units in erythrocyte membranes rather than the actual activity in vivo. The \([\text{H}]\text{ouabain-binding capacity similarly gives a measure of the number of pump units. There is a close correlation between Na\(^{+},K^{+}\)-ATPase activity and \([\text{H}]\text{ouabain-binding capacity} (E. J. Rubython, unpublished observations).}

In the human erythrocyte the majority of total unidirectional efflux of sodium is ouabain-sensitive and therefore in a steady state the unidirectional influx rate of sodium will equal the ouabain-sensitive efflux rate of sodium \((^{\text{d}}M^{\text{Na}}_{\text{Na}})\). The ouabain-sensitive efflux rate of sodium is the product of the ouabain-sensitive efflux rate constant of sodium and the erythrocyte sodium content:

\[
^{\text{d}}M^{\text{Na}}_{\text{Na}} = ^{\text{d}}K^{\text{Na}}_{\text{Na}} \cdot [\text{Na}]_{\text{RBC}}
\]

Thus in a steady state \(^{\text{d}}M^{\text{Na}}_{\text{Na}}\) is a measure of the permeability of the erythrocyte membrane to sodium and \(^{\text{d}}K^{\text{Na}}_{\text{Na}}\) is a measure of the activity of the 'sodium pumps' [6]. A decreased erythrocyte sodium content could result from either a decreased membrane permeability to sodium or increased activity of the 'sodium pumps'.

The results in Table 2 show that the group with the lower erythrocyte sodium content (group A) had a higher activity of the 'sodium pumps' \((^{\text{d}}K^{\text{Na}}_{\text{Na}})\) with a normal efflux rate \((^{\text{d}}M^{\text{Na}}_{\text{Na}})\). This would suggest that the decreased erythrocyte sodium content was due to increased activity of the 'sodium pumps'. The activity of the 'sodium pumps' is determined by the total number of pump units and their degree of activation [6]. In this subgroup \([\text{H}]\text{ouabain-binding capacity} and Na\(^{+},K^{+}\)-ATPase activity were not raised. We have argued that the \([\text{H}]\text{ouabain-binding capacity}\) and the Na\(^{+},K^{+}\)-ATPase activity measurements are indicators of the number of pump units. Thus in this subgroup the activity of each sodium pump unit is increased rather than the total number of pump units.

In group B \((\text{Na}^{+})_{\text{RBC}} > 4\) mmol/kg of cells) the mean plasma urea concentration was higher, indicating a more severe degree of renal failure. In this group the \(^{\text{d}}K^{\text{Na}}_{\text{Na}}\) was lower than that of group A, and we would have expected erythrocyte sodium concentration to be much higher (7-40 mmol/kg of cells instead of 5-47 mmol/kg of cells). However, the ouabain-sensitive efflux rate of sodium \((^{\text{d}}M^{\text{Na}}_{\text{Na}})\) in this group was lower compared with that in healthy subjects. Thus the observed erythrocyte sodium content of 5-47 mmol/kg of cells rather than the expected value of 7-40 mmol/kg of cells is probably explained by a reduction in membrane permeability to sodium.

The results suggest that during the progression of chronic renal failure there is initially an increase in the activity of the 'sodium pumps' (without an increase in the number of pump units), causing a decrease in erythrocyte sodium content. This is probably due to the presence of an inhibitor or the absence of an inhibitor in the plasma, or due to configurational or compositional change in the sodium pump environment.

With further deterioration in renal function, the activity of the 'sodium pumps' as well as membrane permeability decrease so that erythrocyte sodium content becomes nearly normal.

Acknowledgments

We thank Dr A. M. Davison for permission to study patients under his care and Professor D. B. Morgan for helpful advice and criticism during the study.

References


