Leucocyte sodium transport in uraemia

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(Received 7 January 1975)

Summary

1. In sixteen patients with severe chronic renal failure the rate constant for total sodium efflux from leucocytes was significantly reduced compared with that in thirty control subjects. This difference lay chiefly in the glycoside-sensitive ('active') moiety of sodium efflux.

2. In sixteen patients receiving regular haemodialysis, the rate constant for total sodium efflux from the leucocyte was significantly greater than in the undialysed uraemic patients though still subnormal.

3. In individual patients, an increase in sodium efflux could be detected as early as 1 week after regular haemodialysis was started.

4. These results are compatible with the existence of a dialysable molecule in uraemic plasma affecting leucocyte sodium transport.

Key words: leucocyte, sodium transport, uraemia.

Introduction

In some uraemic patients the sodium content of erythrocytes is elevated and this is associated with a defect in the active transport system responsible for sodium extrusion from this cell (Welt, Sachs & McManus, 1964; Welt, Smith, Dunn, Czerwinski, Proctor, Cole, Balfe & Gitelman, 1967). Such abnormalities of sodium transport are likely to affect the regulation of cell water content and therefore cell volume. As uraemia impairs the function of many organs, it is pertinent to ask whether the sodium transport defect is limited to the erythrocyte.

We have recently reported that, in uraemia, the potassium content of the leucocyte is reduced whereas the sodium and water contents are increased (Patrick, Jones, Bradford & Gaunt, 1972; Patrick & Jones, 1974). These findings imply disturbances of cation transport across the leucocyte cell membrane in advanced renal failure. In this paper we describe abnormalities of sodium efflux from the leucocyte in uraemia.

Method

Observations were made on sixteen patients (twelve males, four females; average age 35 years) with chronic renal failure due to a variety of diseases. Plasma creatinine concentrations on the day of the study ranged from 0.96 to 2.35 mmol/l with a mean value of 1.47 mmol/l. Sixteen patients on regular haemodialysis for periods varying from 1 week to 4 years were also studied (ten males, six females; average age 30 years). All patients were ambulant.

The control values for leucocyte electrolyte content were derived from fifty-nine measurements made on hospital staff and students. The control values for leucocyte sodium efflux were based on measurements made on thirty healthy volunteers (average age of all normal subjects was 25 years).

Leucocyte sodium and water contents were determined with techniques that have been described previously (Patrick et al., 1972; Baron & Ahmed, 1969; Boyum, 1968). Preliminary separation of the leucocytes from 50 ml of blood was performed by layering the blood on to a mixture of Hypaque + methyl cellulose of density 1.08. Under these conditions, the erythrocytes were aggregated by the
methyl cellulose and collected in the lower layer. The leucocyte-rich supernatant was then separated and the remaining erythrocytes were removed by carefully controlled hypotonic lysis (Baron & Ahmed, 1969).

Leucocytes prepared as above were also used for the study of sodium efflux as described by Hilton & Patrick (1973). For this purpose the cells were incubated at 37°C for 20 min in a tissue culture medium (TC199, Burroughs Wellcome) to which had been added 5 µCi of 22NaCl. The sodium and potassium concentrations were adjusted to 136 mmol/l and 6.0 mmol/l respectively for both the determination of leucocyte composition and sodium efflux rate constant. This suspension was then centrifuged at 160 g for 3 min, the cells were washed by the addition of 10 ml of TC199, centrifuged again, separated from the extracellular fluid and resuspended in 10 ml of TC199 at 37°C. This suspension was divided into two equal portions and ouabain was added to one portion to give a final concentration of 1.4 µmol/l. Aliquots of cells from both

### Table 1. Rate constants for both total and ouabain-sensitive leucocyte sodium efflux

Results are given for sixteen patients with chronic renal failure before haemodialysis (CRF) and in sixteen patients receiving regular haemodialysis treatment (RDT). Patients 8-16 were studied both before and after the start of RDT. Leucocyte sodium and water contents for the CRF group are also given. In the CRF group there were significant reductions in the rate constants for both total ($P<0.001$) and ouabain-sensitive ($P<0.001$) sodium efflux compared with the control group. In the RDT group there were significant increases in the rate constants for both total ($P<0.001$) and ouabain-sensitive ($P<0.001$) sodium efflux compared with the CRF group. The values given for leucocyte Na and water contents were obtained from the undialysed patients; there were significant increases in both Na ($P<0.05$) and water ($P<0.01$) contents compared with normal subjects.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Plasma creatinine (mmol/l)</th>
<th>Chronic renal failure (undialysed) Leucocyte composition</th>
<th>Chronic renal failure (undialysed) Na efflux rate constant (h⁻¹)</th>
<th>Regular haemodialysis Na efflux rate constant (h⁻¹)</th>
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<tr>
<td></td>
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<td>Na (mmol/kg d.s.) Water (l/kg d.s.)</td>
<td>Total Ouabain-sensitive</td>
<td>Total Ouabain-sensitive</td>
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<tr>
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<tr>
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<td>123 2.98</td>
<td>2.1 1.6</td>
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<td>2.2 1.7</td>
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<td>125 3.07</td>
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<td>± 0.05</td>
<td>± 0.09 ± 0.14</td>
<td>± 0.12 ± 0.13</td>
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<td>Mean 104</td>
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<td>4.0 3.0</td>
<td></td>
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<tr>
<td></td>
<td>sem ± 3.7</td>
<td>± 0.09</td>
<td>± 0.09 ± 0.09</td>
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portions were plunged into crushed ice and centri-
fuged at 0°C at timed intervals up to 12 min from the
addition of the ouabain.

The extracellular fluid was removed from the cell
plugs as completely as possible and the plugs were
counted for radioactivity in a well-type scintillation
counter and scaler (Tracerlab). The dry weights of
the specimens were determined by heating to con-
stant weight at 100°C.

The rate constant for sodium efflux was calculated
from the slope of the regression of the logarithm of
cell radioactivity per unit dry weight against time.
This constant is a measure of the fraction of the
intracellular sodium pool extruded from the cell in
unit time.

From these data it is not legitimate to calculate
sodium efflux from the leucocyte (the product of the
intracellular sodium content and the efflux rate
constant). This is because the intracellular sodium
was measured at room temperature whereas the rate
constant was derived from measurements on cells at
37°C.

Fig. 1. Rate constants for total leucocyte sodium efflux in the
control group, in non-dialysed uraemic patients (CRF) and in
patients receiving regular haemodialysis (RDT). The mean
(±SEM) of the CRF group is significantly reduced ($P<0.001$)
compared with the control group and the mean of the RDT
group is significantly higher ($P<0.001$) compared with the
CRF group.

Fig. 2. Rate constants for total leucocyte sodium efflux before
and after the start of regular haemodialysis in the same
patients (nos. 8–16).

Fig. 3. Time-course of the increase in rate constant for total
leucocyte sodium efflux after the start of haemodialysis
therapy (▲, patient 10; ●, patient 15).
Results
Leucocyte Na\(+\)H\(_2\)O contents were increased in the uraemic patients (Table 1). The rate constant for total sodium efflux was reduced in every patient with uraemia. This was due mainly to a fall in the ouabain-sensitive portion of the flux. The rate constants for total sodium efflux in the dialysed group were significantly higher than in the uraemic group, although they were still subnormal (Fig. 1). Ten patients were studied in both advanced uraemia and after a period of regular haemodialysis. The rate constant for total sodium efflux invariably rose after starting dialysis therapy (Fig. 2) and this effect was evident as early as 1 week after starting treatment (Fig. 3).

Discussion
The high potassium and low sodium content of most mammalian cells (relative to the extracellular fluid) are thought to result from limited permeability of the cell membrane to these cations, together with active transport mechanisms for moving sodium and potassium against their electrochemical gradients. These mechanisms, usually termed cation 'pumps', have in man been studied chiefly in the erythrocyte because of its availability and convenience. However, the erythrocyte is a highly specialized cell, lacking a nucleus, with predominantly anaerobic metabolism and containing the unique protein haemoglobin, which itself influences the cell potassium content (Maizels, 1936).

The leucocyte has a more representative metabolism and much higher transport rates for sodium and potassium than the erythrocyte (Hilton & Patrick, 1973). It might therefore be expected that the leucocyte would be more sensitive to electrolyte and metabolic derangements and this has been found in potassium depletion (Patrick & Bradford, 1972; Edmondson, Thomas, Hilton, Patrick & Jones, 1974) and in renal failure (Patrick et al., 1972; Patrick & Jones, 1974).

In the uraemic patients we describe, the rate constant for total sodium efflux from the leucocyte was reduced in every case, and the defect was confined to the ouabain-sensitive portion. Although the sodium efflux rate constant did not correlate with plasma creatinine, creatinine clearance or blood urea, uraemia was advanced in these patients and such correlations might emerge from study of a wider range of renal function.

Cole, Balfe & Welt (1968) have shown that normal erythrocytes incubated in uraemic plasma develop a defect in ouabain-sensitive membrane adenosine triphosphatase, an enzyme which appears to be intimately associated with the working of the sodium pump. Thus it seems likely that an unidentified chemical with the capacity to inhibit the energy supply of the sodium pump is circulating in the plasma of uraemic patients. Bricker (1972) has argued that the factor depressing sodium transport is involved in the reduction of the tubular sodium reabsorption characteristic of chronic renal failure.

Our observations imply that a defect in sodium transport may be widespread in uraemia. The rapidly beneficial effects of haemodialysis on the transport defect of uraemic patients which we have demonstrated in the leucocyte are compatible with a role for a dialysable molecule in the production of this defect.

References


