Leucocyte ATP and renal failure

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Summary

1. We have measured adenosine 5'-triphosphate (ATP) levels in isolated leucocyte suspensions from patients with renal failure.

2. Leucocyte ATP is reduced in these patients, unlike erythrocyte ATP which is known to be elevated in uraemia.

3. The relevance of reduced leucocyte ATP to the abnormalities of cation transport and cell function which occur in uraemia is discussed.

Key words: adenosine 5'-triphosphate, leucocyte, uraemia.

Introduction

Increased cell sodium content and reduced active transport of sodium across the cell membrane are found both in erythrocytes [1] and leucocytes [2, 3] from uraemic patients. Sodium transport in renal failure is also depressed in brain [4], renal cortex [5] and intestinal mucosa [6]. Such widespread changes in sodium transport, which is an important determinant of cell volume, could have major consequences for cell and organ function in uraemia. It has been suggested that they are caused by a secondary 'spill-over' effect of a circulating natriuretic factor (or factors) thought to underlie the adaptive changes in sodium transport characteristic of nephrons still functioning in diseased kidneys [7]. If so, the changes in sodium pump activity might result from a common biochemical abnormality, such as a limitation of the availability of adenosine 5'-triphosphate (ATP) for cation transport. In this context in uraemia erythrocyte ATP is considerably increased and the activity of membrane 'transport' adenosine triphosphatase [(Na+,K+-activated)-ATPase] in erythrocyte ghosts is reduced [1, 8].

Several factors may cause elevation of erythrocyte ATP in uraemia [9] and it is important to determine whether this change is peculiar to the erythrocyte or whether it is also found in other cells with impaired sodium transport in renal failure. This paper describes the changes in leucocyte ATP content observed in patients with acute and chronic renal failure and the effects of haemodialysis.

Methods and patients

Leucocytes were isolated from normal blood by dextran sedimentation [10] and from uraemic blood by methylcellulose/Hypaque layering [11] as previously described. Washing stages were performed with TC 199 medium containing 15 μmol of elemental zinc/l and the cells were incubated at 37°C for 30 min before disruption by perchloric acid followed by snap-freezing. The ATP content of the supernatant was determined by a modification of the phosphoglycerate kinase (EC 2.7.2.3) method [12] with reagents supplied by Boehringer (kit no. 123897). Results are expressed as nmol of ATP/10^6 leucocytes.

Leucocyte ATP was determined in 30 healthy control subjects, 12 patients on maintenance haemodialysis and 14 undialysed patients with stable chronic renal failure, with serum creatinine ranging from 250 to 1700 μmol/l. Eight patients with acute renal failure, all of whom displayed one or more of the indications for emergency dialysis [13], were also studied at presentation and after periods of treatment (dialysis, seven patients; intravenous resalination, one patient) varying from 4 to 30 days.

Results

The leucocyte suspension was found to contain no erythrocytes, with a platelet/leucocyte ratio of less than 1:100, and there was no significant
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The difference in the percentage of polymorphonuclear leucocytes in suspensions prepared from normal controls (75 ± 3.4%, mean ± SEM, n = 10) and uraemic subjects (82 ± 2.4%, n = 14). Leucocyte morphology by light and scanning electron microscopy was normal and trypan blue exclusion was greater than 99%. Leucocyte clumps were absent as shown by stained films and by the observation that cell counts on duplicate samples from the same suspensions agreed closely (16 323 ± 899 vs 16 597 ± 921 cells/μl, mean ± SEM, n = 29, P = N.S.). Overall leucocyte recovery was about 50%, recovery of a known amount of ATP was 93 ± 3% (mean ± SEM, n = 10) and ATP in the frozen acidified cell extract was stable for at least 4 days. Although TC 199 medium is originally constituted with 5 mg of ATP/l it was found that the ATP level declined rapidly with storage so that the TC 199 medium was in fact free of ATP.

Leucocyte ATP in 30 normal subjects was 2.26 ± 0.06 nmol/10^6 cells (mean ± SEM). There was no significant difference between the ATP content of normal leucocytes isolated by dextran sedimentation (2.20 ± 0.11 nmol/10^6 cells, mean ± SEM, n = 10) or methylcellulose/Hypaque layering (2.26 ± 0.12 nmol/10^6 cells, mean ± SEM, n = 10). Leucocyte ATP was significantly reduced compared with that of normal control subjects both in patients with untreated uraemia (1.77 ± 0.07 nmol/10^6 cells, mean ± SEM, n = 14, P < 0.01) and those on maintenance haemodialysis (2.02 ± 0.05 nmol/10^6 cells, mean ± SEM, n = 12, P < 0.05).

In patients with untreated uraemia leucocyte ATP tended to fall progressively as the serum creatinine concentration increased above 700 μmol/l, and there was also a significant negative correlation (r = -0.76, P < 0.005) between the serum phosphate concentration and leucocyte ATP.

Leucocyte ATP was also reduced in patients with acute renal failure (1.48 ± 0.05 nmol/10^6 cells, mean ± SEM, n = 8; Fig. 1) and a significant increase towards normal values (1.97 ± 0.09 nmol/10^6 cells, mean ± SEM, P < 0.001) occurred after treatment with dialysis or resalination.

There was no correlation (r = 0.12, P = N.S.) between leucocyte ATP content and the percentage of polymorphonuclear leucocytes in suspensions from both control subjects and uraemic patients and mixing experiments showed no evidence that the experimental observations resulted from a direct inhibitory effect on the ATP assay of the leucocyte extract from uraemic patients.

**Discussion**

The normal range for leucocyte ATP found in this study is greater than that reported by previous authors [14—16]. Low ATP levels would be predicted from the absence of an incubation stage, to allow metabolic recovery of the cells, in the isolation technique employed by the first two groups [14, 15]. The poor metabolic state of the leucocytes studied by Scholar et al. [15] is reflected in the low ATP/ADP (adenosine 5'-pyrophosphate) ratio (7:1) and energy charge.

![Fig. 1. Effect of treatment (dialysis or resalination; duration (days) shown by the numbers) on the leucocyte ATP content of patients with acute uraemia. One patient (O—O) was treated by intravenous resalination. Results are expressed as means ± SEM; symbols on the left represent the leucocyte ATP content before (*1.48 ± 0.05, mean ± SEM, n = 8) and those on the right after (**1.97 ± 0.09) treatment.](image-url)
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(0.87). For leucocytes isolated by the method we used these values are 13:1 and 0.95 respectively, determined by high-pressure liquid chromato-

graphy. This technique gave values for normal leucocyte ATP (n = 5) of 1.89 ± 0.2 nmol/10^6 cells (mean ± SEM), which is not very different from that found with the enzyme assay for ATP (M. A. Mansell, R. A. Harkness & R. J. Simmonds, unpublished work). A previous study [17] reported a much higher normal range although no details of isolation or analytical method were given.

Although nucleotides other than ATP can function as substrates for phosphoglycerate kinase, their effect relative to ATP is small; even at saturating concentrations guanosine 5'-triphosphate (GTP) is only 50% as effective on a molar basis as ATP [18]. The GTP/ATP ratio in leucocytes is less than 1:3 and is unaltered in the presence of renal failure (D. Perrett & M. A. Mansell, unpublished work) so that the maximum error of the present method attributable to this source is an overestimate of leucocyte ATP by 15%.

The tendency of leucocyte ATP to vary inversely with the serum creatinine concentration and the rapid response to haemodialysis resemble the changes in leucocyte sodium transport described by Edmondson et al. [3], suggesting a relationship between the two. In the erythro-
cyte the K_m for ATP of membrane (Na^+, K^+-activated)-ATPase is about half the intra-
cellular ATP level [19, 20]. If a similar relation-
ship exists in leucocytes then the degree of reduction of leucocyte ATP found in the present study would be sufficient to limit the transport rates attainable by the sodium pump. The metabolic parallels between uraemia and mal-
nutrition [21] and the finding of diminished ATP levels in the leucocytes of malnourished children [14] lend further support to the concept of diminished energy supply at a cellular level in renal failure.

Alternatively reduced ATP levels might result from inhibition of the sodium pump. Active-
cation transport is a key determinant of cellular energy consumption [22] and Atkinson [23, 24] has suggested that the activities of the ATP-regenerating enzymes are regulated by intra-
cellular ATP or a related function such as energy charge. This work has shown that both are reduced in uraemic leucocytes. In this context it is of interest that an increase in the concentration of extracellular zinc not only stimulates the leucocyte sodium pump [25], but also leads to increased ATP levels in these cells (M. A. Mansell & N. F. Jones, unpublished work).

Whatever the cause, reduced availability of ATP may explain some of the abnormalities of leucocyte function found in uraemia [9], possibly by reducing the supply of phosphoribosylpyrophosphate for purine synthesis de novo [26]. Further studies of purine metabolism in uraemia are in progress and may eventually provide an explanation for the opposite changes in ATP levels found in erythrocytes and leucocytes in this condition.

References

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