**SHORT COMMUNICATION**

**Effect of zinc on leucocyte sodium transport in vitro**

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**Summary**

1. In a preparation of human leucocytes maintained in tissue culture fluid, increasing the extracellular zinc concentration leads to a significant increase in both ouabain-sensitive sodium efflux and sodium influx.

2. Cell water and sodium content do not alter significantly with increasing extracellular zinc concentration.

3. A small increase in the ouabain-insensitive sodium efflux can be demonstrated when the external zinc concentration is raised from 0.75 μmol/l to 90 μmol/l.

**Key words:** leucocyte, sodium, transport, zinc.

**Introduction**

We have previously reported the use of human leucocytes in the investigation of disorders of cation transport and intracellular composition. During a study of the effect of malnutrition on leucocyte sodium transport, an increase in the rate constant for sodium efflux was noticed at a time when zinc was added to the diet. Low rate constants for leucocyte sodium efflux have been reported in uraemia, hypertension and malnutrition (Edmondson, Hilton, Jones, Patrick & Thomas, 1975a; Edmondson, Thomas, Hilton, Patrick & Jones, 1975b; Patrick, 1975), diseases which have also been reported to be associated with low plasma zinc concentrations (Mansouri, Halstead, Sukry, Prasad, Gabre, Elmifrey, Monkhatar & Darby, 1965). Our studies are conducted on leucocytes incubated in tissue culture fluid that has a variable zinc concentration (TC 199, Burroughs Wellcome), and we have therefore explored the possibility that alteration of the extracellular zinc concentration might influence sodium transport. This paper reports the effects of the extracellular concentration of zinc on sodium fluxes and univalent cation content of normal human leucocytes in vitro.

**Methods**

**Preparation of leucocytes**

Leucocytes were prepared from healthy volunteer subjects by a previously reported modification (Hilton & Patrick, 1973) of the method of Baron & Ahmed (1969). The cell preparation was divided into equal portions and then suspended in tissue culture medium (TC 199, Burroughs Wellcome) identical in every respect apart from the zinc concentration, which was varied from 0.75 μmol/l (5 μg/100 ml) to 90 μmol/l (600 μg/100 ml). The sodium and potassium concentrations of the media were 136 and 6.0 mmol/l respectively and the pH was in the range 7.35–7.45. The studies were conducted at 37°C after 30 min incubation, by which time the sodium, potassium and water content of the cells have reached equilibrium.

**Sodium efflux and influx**

**Efflux.** The rate constant for sodium efflux was determined as previously described (Hilton & Patrick, 1973). Experiments were performed with
batches of leucocytes exposed to tissue culture medium with zinc concentrations of 0-75, 15, 45 and 90 μmol/l (5, 100, 300 and 600 μg/100 ml). The sodium efflux rate constant was determined both in the presence and absence of a maximally inhibitory concentration of ouabain (1-4 μmol/l; 10 μg/ml). At this concentration the inhibition of sodium transport is virtually instantaneous. The difference between these two rate constants represents the ouabain-sensitive component of sodium efflux. We were not able to study leucocytes in an entirely zinc-free medium as TC 199 normally contains a small quantity of zinc (0-6-1.0 μmol/l) derived from the amino acid component.

Influx. Cells in which sodium influx was to be studied were incubated for 30 min in media of the same composition as those used for the study of sodium efflux and the 22Na influx was observed over 8 min after the addition of 3 μCi of 22NaCl to 4 ml of the cell suspension. Sodium influx was measured simultaneously in batches of cells exposed to media of differing zinc concentrations, in the presence and absence of ouabain, and corrected for simultaneous efflux according to methods previously described (Hilton & Patrick, 1973).

Sodium content

Leucocyte sodium and water content were studied after exposure of portions of the original cell suspensions to media of differing zinc concentrations for 30 min at 37°C. The method for the estimation of cell composition was according to the technique of Baron & Ahmed (1969), except that the cells were washed once at 0°C in MgCl2 containing 51 Cr-labelled EDTA as a marker of extracellular fluid contamination.

Results

Results are shown as mean values ± SEM.

Sodium efflux

The rate constant for sodium efflux was studied in 50 experiments. The results are shown in Fig. 1. The total sodium efflux rate constant is increased by exposure to higher concentrations of extracellular zinc, the most marked change occurring between the medium with the nominally zero zinc concentration (0-75 μmol/l) and that with a zinc concentration of 15 μmol/l. The difference between the values at each external zinc concentration was significant (P < 0.05). No significant change could be shown in the ouabain-insensitive efflux rate constant between the media containing 'zero' zinc and zinc at a concentration of 15 μmol/l. However, there was a difference between the ouabain-insensitive component of sodium efflux of 0.52 ± 0.06 at a zinc concentration of 0-75 μmol/l and 0.77 ± 0.06 at a zinc concentration of 90 μmol/l (P < 0.02). The difference between the total and ouabain-insensitive rate constants, i.e. the ouabain-sensitive portion of sodium efflux, rose significantly with each increment in the external zinc concentration (P < 0.05).

Sodium influx

The effect on sodium influx of raising the external zinc concentration was studied in 40 experiments and the results are also shown in Fig. 1. Sodium influx (mmol of Na h⁻¹ kg⁻¹ dry cell weight) is stimulated by increasing the external zinc concentration in a manner similar to the efflux rate constant. There was a significant difference, on a paired basis, with each increment of zinc concentration apart from the last (P < 0.02). No ouabain-sensitive sodium influx could be demonstrated at any of the external zinc concentrations studied.

Leucocyte composition

Twenty-one experiments were performed in which the sodium and water content of leucocytes were compared at different external zinc concentrations. Cell water did not differ significantly over the range of concentrations studied (2.62 ± 0.06 l/kg dry weight at [Zn] 0-75 μmol/l, and 2.70

![Fig. 1. Leucocyte total sodium efflux rate constant (•) and sodium influx (■) according to the extracellular concentration of zinc.](image-url)
Zinc and leucocyte sodium transport

Discussion

We have demonstrated that changes in external zinc concentration result in changes in transmembrane sodium transport rates in human leucocytes studied in tissue culture medium in vitro. The size of the changes in the sodium efflux rate constant and in sodium influx are similar and no net change in intracellular sodium or water content can be demonstrated.

These studies provide little information as to the mechanism underlying the changes in sodium transport and any suggestions are necessarily speculative. It is not possible to regard the observations as representing simple changes in the sodium pump itself. The ouabain-sensitive component of sodium efflux, which is conventionally equated with the sodium pump, is increased with increasing concentration of external zinc, without any change in the intracellular sodium content. However, the increment in sodium influx is not ouabain-sensitive and is thus unlikely to be a reflection of sodium:sodium exchange via the sodium pump. Thus, presumably, the increased sodium influx takes place through a mechanism other than the sodium pump. The small increase in the ouabain-insensitive component of sodium efflux, at high external zinc concentrations, also points to a membrane effect independent of the sodium pump. The addition of zinc to the extracellular fluid affects sodium transport if more cell membrane per unit cell mass had been exposed, with deployment of more sites for the entry and exit of sodium from the cell. We have, however, not demonstrated any alteration in cell volume, as measured by cell water, and morphologically there is no difference between cells incubated in the different media.

Both the sodium pump and membrane integrity are closely dependent on the supply of energy in the form of ATP. It is therefore possible that the effect of zinc, in our preparation, is related to an effect on the energy supply. Zinc is known to form complexes with ATP in a manner similar to magnesium, though such complexes have generally been considered to make ATP less, rather than more, available for metabolic processes. The role of zinc in the intracellular compartment is poorly understood and, although it is known to be an essential cofactor for a number of enzymes, none of these appears to be directly connected with sodium transport.

The concentration of zinc in our nominally zero zinc medium was 0.75 μmol/l, and substantial increases in sodium transport were seen on raising the concentration of zinc to 15 μmol/l, approximately the concentration in normal plasma. However, it cannot necessarily be assumed that tissue culture fluid with a zinc concentration of 15 μmol/l (100 μg/100 ml) is directly comparable to plasma in this respect. Plasma zinc is extensively bound to protein (albumin, transferrin and α1-macroglobulin) and the concentration of unbound zinc is approximately the same as in our low zinc medium. It is, however, clear that zinc is capable of altering membrane transport, in the leucocyte at least, and that its concentration needs to be controlled in studies with this isolated cell preparation.

References


