Intracellular Na\(^+\) and Ca\(^{2+}\) activities in essential hypertension

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Summary
1. The intracellular Na\(^+\) and Ca\(^{2+}\) activity and Na\(^+\) concentration were measured in erythrocytes of normotensive subjects, with and without a familial disposition to hypertension, in essential hypertensive patients with and without a family history of hypertension, and in patients with secondary hypertension.

2. In normotensive subjects without a genetic trait of hypertension intracellular Na\(^+\) activity and concentration were \(7.00 \pm 1.38\) mmol/l and \(5.67 \pm 0.95\) mmol/l respectively. The intracellular Ca\(^{2+}\) activity was \(4.82 \pm 4.49\) \(\mu\)mol/l. In normotensive subjects with a familial hypertensive disposition intracellular Na\(^+\) activity and concentration were \(9.74 \pm 1.43\) mmol/l \((P < 0.01)\) and \(6.63 \pm 0.88\) mmol/l \((P < 0.05)\). Intracellular Ca\(^{2+}\) was \(9.59 \pm 9.71\) \(\mu\)mol/l \((P < 0.05)\).

3. Essential hypertensive patients without a familial genetic trait had an elevated intracellular Na\(^+\) activity \((8.35 \pm 2.08\) mmol/l, \(P < 0.05)\). Intracellular Na\(^+\) concentration was \(6.64 \pm 0.79\) mmol/l \((P < 0.05)\). The intracellular Ca\(^{2+}\) activity was markedly elevated to \(25.33 \pm 19.03\) \(\mu\)mol/l \((P < 0.01)\). The essential hypertensive patients with a familial disposition had an elevated intracellular Na\(^+\) activity \((17.19 \pm 4.37\) mmol/l, \(P < 0.001)\) and Ca\(^{2+}\) activity \((32.8 \pm 32.51\) \(\mu\)mol/l, \(P < 0.01)\). The intracellular Na\(^+\) concentration was \(6.25 \pm 1.23\) mmol/l.

4. The results indicate that in essential hypertension intracellular Na\(^+\) activity is increased, particularly in patients with a familial disposition for hypertension. Intracellular Ca\(^{2+}\) is increased in essential hypertension whether or not there was a family disposition to hypertension.

Key words: calcium, erythrocytes, sodium.

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Introduction
Since the discovery by Losse et al. in 1960 [1] that in essential hypertension there is a generalized disturbance of the transmembrane Na\(^+\) distribution, many attempts have been made to clarify the nature of the underlying cellular defect. However, the results on transmembrane ion fluxes in hypertension have remained controversial, as no single transport defect is detectable in essential hypertensive patients [2]. On the other hand, animal experiments suggest that a defective ion binding to intracellular macromolecules plays an important role, as the free fraction of intracellular Na\(^+\) was elevated when the total Na\(^+\) concentration was not [3, 4]. In this study we have examined the changes in the free intracellular Na\(^+\) and Ca\(^{2+}\) concentrations in patients with essential hypertension with and without a family history of essential hypertension, and in the first grade relatives of essential hypertensive patients. In addition patients with secondary hypertension were examined.

Methods
Measurements
The measurements of intracellular Na\(^+\) concentration, intracellular Na\(^+\) activity and Ca\(^{2+}\) activity were performed in 10 ml of heparinized venous blood. First the blood was three times washed in isotonic MgCl\(_2\) solution. Thereby theuffy coat was removed. Then part of the erythrocyte pellet was partly frozen to \(-18^\circ\)C to haemolyse the cells for the activity measurements. Another part of the pellet was haemolysed by adding 200 volumes of LiCl solution (3 mmol/l) and measurements of concentrations were made by flame photometry. The determination of intracellular Na\(^+\) and Ca\(^{2+}\) activity was made by ion-selective electrodes. For the
Na\(^+\) determination a Na\(^+\) sensitive glass electrode was used. The selectivity coefficient of this electrode against K\(^+\) was in the range of 10\(^{-3}\). The Ca\(^{2+}\) measurements were performed with a PVC membrane containing a Ca\(^{2+}\) selective neutral ligand, which was kindly supplied by Professor Simon, ETH Zürich, Switzerland. The Ca\(^{2+}\) electrode had a selectivity against K\(^+\) in the range of 5 \times 10\(^{-6}\) (mol/l)\(^{-1}\). According to this value Ca\(^{2+}\) can be reliably detected down to 10\(^{-8}\) mol/l. As reference electrode an Ag/AgCl wire was stuck into a small plastic tube through which flowed KCl solution (100 mmol/l) at a constant rate of 150 \(\mu\)l/h. The proper arrangement of the reference system proved to be of utmost importance, since shifts of the potential at the reference electrode can be elicited by precipitation of the proteinaceous intracellular fluid at the surface of the reference electrode. Both Na\(^+\) and Ca\(^{2+}\) electrodes showed the theoretically expected slopes of 58 and 29 mV respectively.

**Patients**

The investigations were performed in 16 essential hypertensive patients with a family history of hypertension, in 10 essential hypertensive patients without a known familial disposition, and in 31 normotensive subjects with at least one hypertensive parent, and in 59 normotensive subjects with no family history of hypertension. The group of secondary hypertensive subjects consisted of one patient with Conn syndrome, one with Cushing’s disease, one with hypercalcaemia and one with renal hypertension. All patients studied had neither dietary nor pharmacological antihypertensive treatment at the time of the study or at least 6 weeks before the investigation. Furthermore none of them was on digitalis, which is known to increase intracellular Na\(^+\) markedly.

**Results**

The mean value and standard deviation of intracellular Na\(^+\) activity in normotensive subjects without a familial disposition for hypertension was 7.00 \pm 1.38 mmol/l. The intracellular Na\(^+\) concentration in this group was 5.67 \pm 0.95 mmol/l (Fig. 1). These subjects had an intracellular Ca\(^{2+}\) activity of 4.82 \pm 4.45 \(\mu\)mol/l. In normotensive subjects with a proven family history of hypertension the intracellular Na\(^+\) activity was raised to 9.74 \pm 1.43 mmol/l (P < 0.01). Similarly the intracellular Na\(^+\) concentration and Ca\(^{2+}\) activity were slightly elevated to 6.63 \pm 0.88 mmol/l and 9.59 \pm 9.71 \(\mu\)mol/l (P < 0.05). However, there was still a considerable overlap, especially between the Ca\(^{2+}\) values in both groups. The secondary hypertensive patients all had a normal intracellular Na\(^+\) activity (7.68 \pm 1.29 mmol/l) and intracellular Na\(^+\) concentration (6.18 \pm 1.46 mmol/l). The intracellular Ca\(^{2+}\) activity was inhomogeneous in the patients.

The essential hypertensive patients without a family history of hypertension had also a slightly raised intracellular Na\(^+\) activity (8.35 \pm 2.08 mmol/l, P < 0.05) and concentration (6.64 \pm 0.79 mmol/l, P < 0.05). The range of intracellular Na\(^+\) activity was wider in this group than in the non-disposed normotensive subjects. The intracellular Ca\(^{2+}\) activity was clearly elevated, as opposed to the normotensive subjects (25.33 \pm 19.03 \(\mu\)mol/l, P < 0.01). The essential hypertensive patients with a familial disposition for hypertension had a distinctly elevated Na\(^+\) activity (17.19 \pm 4.37 mmol/l, P < 0.001). The intracellular Na\(^+\) concentration was only slightly raised (6.25 \pm 1.23 mmol/l). There was also a markedly raised intracellular Ca\(^{2+}\) activity (32.8 \pm 32.51 \(\mu\)mol/l, P < 0.01). Generally the hypertensive groups had a wider variation of intracellular Ca\(^{2+}\) activity than the normotensive subjects.

**Discussion**

In essential hypertensive patients there is an elevation of intracellular Na\(^+\). This finding of elevation of intracellular Na\(^+\), which was first described by Losse et al. [1, 5, 6] and has since often been confirmed, has been extended to other blood cells such as lymphocytes and polymorphonuclear leucocytes [7-10]. Furthermore it became evident that antihypertensive treatment may lower intracellular Na\(^+\) so that a
clear difference from normotensive subjects may not be detectable [11].

The present results suggest that the increase in intracellular Na\(^+\) is greater in Na\(^+\) ionic activity than in total Na\(^+\) concentration. Furthermore there seems to be a difference between those essential hypertensive patients with and those without a familial disposition for hypertension. In human essential hypertension there might therefore be a defective binding of Na\(^+\) to intracellular proteins. In some patients the Na\(^+\) activity is severalfold the Na\(^+\) concentration. This is due to the fact that the Na\(^+\) activity is referred to the respective volume of free water as opposed to the Na\(^+\) concentration, being expressed as mmol/l of cell volume. The water content of the erythrocytes has been determined to be about 60%. However, a certain fraction of water molecules is required to form hydration spheres around ions and ionic groups of the macro-molecular matrix and only 50% of the cell water may be free [12].

The intracellular Ca\(^{2+}\) activity also was increased in essential hypertension, but this increase was not specific for essential hypertension. Furthermore in an earlier study in eight renal hypertensive patients a slightly elevated intracellular Ca\(^{2+}\) activity was found [13]. Thus it seems possible that a raised intracellular Ca\(^{2+}\) activity may be the common denominator in a variety of hypertensive states, whereas the intracellular Na\(^+\) activity probably reflects the presence of a genetic disposition for essential hypertension.

The intracellular Ca\(^{2+}\) activity showed large variations and it is possible that the procedure of freezing and destroying the cells altered the binding properties of the cytoplasm and the membranes. The measured intracellular Ca\(^{2+}\) activity may not reflect the values in vitro. Studies with Ca\(^{2+}\) antagonists in hypertensive subjects suggest that erythrocyte Ca\(^{2+}\) metabolism may be less influenced by changes of transmembraneous Ca\(^{2+}\) fluxes than that of smooth muscle cells [13].

The present study shows that in essential hypertension intracellular Na\(^+\) metabolism is invariably disturbed and suggests than an elevation of free intracellular Ca\(^{2+}\) occurs, which may contribute to the manifestation of hypertension.

References