ERYTHROCYTE SODIUM-POTASSIUM CO-TRANSPORT IN HYPERTENSION

D de Zeeuw, J F Jilderda, T Tepper

State University Hospital, Gronigen, The Netherlands

Summary

This paper reports on the measurement of sodium-potassium co-transport across red cell membranes in patients with hypertension and in normotensive volunteers. No differences were found in flux values between these two groups. Anti-hypertensive medication such as propranolol and enalapril (converting enzyme inhibitor) had no unequivocal effect on the measured cation transport. Individual diurnal and day-to-day variation of fluxes appear to be substantial. It is concluded that uniformity in the assay procedure may be of great importance before studying any difference in cation transport between various populations.

Introduction

The measurement of cation fluxes across cell membranes has become a very popular topic ever since the first data was published on a relation between defective fluxes and essential hypertension (EH). Apart from the scientific value of gathering more data to gain insight into the causes of hypertension, the individual flux values were thought to be of diagnostic importance in discriminating secondary and essential hypertension. The data however became more and more confusing since various investigators found different values for mean cation fluxes in controls as well as in hypertensive patients. Part of these discrepancies could be due to the various modifications that have been made with respect to the assay method. In addition various investigators measure different cation transport mechanisms and in different cells.

We have investigated some of the parameters that could possibly account for these discrepancies. Therefore the sodium-potassium co-transport was studied in patients with EH and in controls. In addition the effect of antihypertensive medication (converting enzyme inhibition vs propranolol) on the fluxes was analysed, and the confidence limits of the gathered sodium-potassium co-transport values were tested.
Patients and methods

Sodium-potassium co-transport was determined in 38 normotensive volunteers (21 \( \sigma \); 17 \( \varphi \)) and in 33 patients with documented EH (17 \( \sigma \), 16 \( \varphi \)). The mean age of both groups was 35 \( \pm \) 12 yr (range 21 to 62 yr) and 47 \( \pm \) 8 yr (range 24 to 63 yr), respectively. None of the patients were overweight. Antihypertensive medication had been withdrawn for at least one month before study. Blood pressure of all subjects was measured at 9.00 a.m. after 10 min of supine rest, and blood samples were drawn subsequently. Twenty-two patients were evaluated again after being on antihypertensive medication for at least 12 weeks. Half of the patients had taken propranolol (ranging from 40 to 120mg twice daily), whereas the other 11 had taken the converting enzyme inhibitor enalapril (5 to 20mg twice daily).

All blood samples (10ml mixed with 0.1ml 10 per cent EDTA) were processed within two hours of collection. Red cells were washed with saline thrice. Then they were incubated for 16 hours at 4°C under gentle rotation at eight per cent haematocrit in a medium containing (mmol/L): 50NaCl, 3KCl, 1EGTA, 200 cholinechloride, 1.25Na\(_2\)PO\(_4\), 1MgCl\(_2\), 1.25Na\(_2\)HPO\(_4\) and 0.01PCMBs (4°C; pH: 7.2). Thus cells were loaded with an equimolar amount of sodium and potassium (35mmol/L cells). Recovery of the red cells was achieved by one hour incubation (37°C) at a haematocrit of 10 per cent in a medium containing (mmol/L): 150NaCl, 1MgCl\(_2\), 5Na\(_2\)HPO\(_4\), 1EGTA, 4 cysteine, three inosine, 2 adenine and 10 glucose (37°C; pH: 7.2). After washing the cells five times, they were diluted to a haematocrit of six per cent with medium containing (mmol/L): 75MgCl\(_2\), 85 sucrose, five glucose, 0.1 ouabain, 10MOPS, one frusemide and Tris was added to reach pH 7.2 (37°C). Samples for measurement of sodium and potassium by Flamephotometry were taken at 0.5, 1, 1.5 and two hours after starting the incubation (37°C). Co-transport of sodium as well as potassium, was calculated from the difference between the slopes of the two linear regression lines that could be derived from the successive concentrations of each cation both when frusemide was absent and present in the incubation medium. All solutions used were freshly prepared on the day of measurement.

Results

Mean blood pressure in the control subjects was 122/74 \( \pm \) 14/11mmHg. The hypertensive group had a mean blood pressure of 164/106 \( \pm \) 17/8mmHg. Of the 22 hypertensives that were followed after medication the first 11 had mean blood pressures of 155/104 \( \pm \) 10/5 before and 141/91 \( \pm \) 15/9mmHg after propranolol, whereas the 11 patients on enalapril were 168/109 \( \pm \) 17/8 and 138/92 \( \pm \) 12/7 mmHg respectively.

Figure 1 shows the individual values of the sodium-potassium co-transport. There appears to be no difference between controls and hypertensives.

Antihypertensive treatment had no consistent effect on cation co-transport: in the propranolol group mean sodium flux (\( \mu \)mol/L/h) was 441 \( \pm \) 201 before and 376 \( \pm \) 188 after therapy, the median change being -5 per cent (range +6 to -64%). The enalapril group also failed to show a significant change: 346 \( \pm \) 163
Sodium efflux
(\text{\textmu}mol.l^{-1}.h^{-1})

Potassium efflux
(\text{\textmu}mol.l^{-1}.h^{-1})

Controls Hypertensives Controls Hypertensives

Figure 1. Individual values of the sodium and potassium co-transport in normotensive controls and patients with hypertension. The horizontal bars represent the mean values before and 334 ± 144\text{\textmu}mol/L/h after therapy, the median change was also −5 per cent with a range from +88 to −37 per cent. Potassium efflux measurements gave similar results including an even greater scatter in the percentage change.

To establish the confidence limits of this co-transport measurement the following tests were performed in six healthy normotensive volunteers: Intra-assay variation was measured as very low. Both for the sodium and potassium flux, the variation coefficient (VC) was less than three per cent in both the high and low range of cation fluxes. The VC within the cation fluxes of the same blood sample determined on two consecutive days was less than five per cent (interassay). However when testing the day-to-day variation in the fluxes of an individual, the VC fluctuated from one to 37; in only two of the six subjects the VC of both sodium and potassium flux was less than seven per cent. In two of these volunteers co-transport was measured in blood samples taken at four different times in the day. Diurnal variation was six per cent in one subject and 22 per cent in the other. There was a tendency to lower fluxes towards the end of the day.

Discussion

A high systemic arterial blood pressure is a very common phenomenon in the western civilisation. The prevalence is estimated to be around 10–15 per cent. In 90 per cent of the subjects with high blood pressure no demonstrable cause will be found by means of the available diagnostic procedures. Thus, extensive
screening is needed to differentiate between EH and secondary hypertension. Since this screening has high costs and low benefits, and since the different diagnostic methods give a varying percentage of false negative or false positive results, the paper of Garay [1] promised an elegant solution to the problem. His data at that time, showed that the ratio of sodium efflux and potassium influx of the erythrocyte was able to differentiate 100 per cent between EH and normotension. However, since then a boom of conflicting data has been published on this topic. Some results point to the ouabain-sensitive cation flux [2], others to the sodium-potassium co-transport [3], or the lithium-sodium counter-transport [4] as a marker of EH. Whatever cell type or flux was studied, some investigators found no distinction between patients with EH and normotensive subjects, whereas others found a significant difference in flux between patients and controls. In the latter studies a considerable overlap between the two groups was still present.

The data of the present study furnish no proof of a difference in the sodium-potassium co-transport between patients with EH and normotensive volunteers. The cation flux values found in our control group agree with those found by others [5,6]. Thus, the difference must be found in the measured co-transport of the hypertensive patients. Garay and others [5] find a lower cation outflow whereas Adragna [7] reports a higher co-transport in the hypertensive population studied. As suggested by this last group these discrepancies could well be due to different sodium and potassium concentrations in the red cells used to assay the co-transport function. Apart from the method, it appears that other factors may well contribute to the differences such as: the selection of patients and control subjects, blood sampling, day-to-day variation, diurnal variations, dietary regimens, and medication. With respect to the selection of subjects it is difficult to assess any differences between the various studies. The previous antihypertensive medication and the time between withdrawal and assay might play a role. This was however, not confirmed by our data on the effects of propranolol and enalapril. We standardised blood sampling with respect to the position of the subject and the time of the day. The latter could be of importance since a diurnal variation in cation fluxes may well be present, according to our preliminary results. Furthermore, the substantial variation of the individual day-to-day flux values has to be taken into account in the evaluation of the flux differences between patients and controls. A dietary regimen such as a restricted sodium intake, which is often advised to a hypertensive patient, may interfere with the assay. In fact Edmondson [8] provided suggestive evidence for a positive correlation between plasma renin activity and (leucocyte) cation transport.

In conclusion, the results of the present study underline the need for a standardised experimental protocol when searching for differences in cation co-transport between hypertensives and controls. This was also suggested by Parker and Berkowitz in a recent review on this topic [9]. The observed individual diurnal and day-to-day variation of red cell cation transport may interfere with the assessment of a difference between groups of subjects. On the other hand, the observed variation might, after further study, lead to a better understanding of the factors that determine the activity of these transport mechanisms.
Open Discussion

LEVER (Chairman) It's my impression that what you have done now, and have still not completed, ought to have been done before anyone did any work whatsoever comparing normotensives and hypertensives. In other words there was no evidence on replicate variation, no studies of diurnal variation and no studies on the effects of previous drug treatment. The introduction of a new method without such ordinary regular checking of the method's validity before the first blast-off with normotensive and hypertensive data has to some extent obscured this potentially very important field. I think your paper is welcome and you are to be congratulated in producing, perhaps belatedly, things which ought to have been done a long time ago.

UNNAMED FROM HOLLAND Did you check whether there is any relation between age and sodium efflux? If there is a relation between sodium efflux and age, the difference in age between the control group and the hypertensives may be critical.

de ZEEUW Yes, you are quite right. We checked that, both in the normotensive population and also in the hypertensive subjects, and there is no correlation within the range of age that we have in our population, that is in people of approximately 20 to 60 years.

LEVER How many patients did you put in that correlation?

de ZEEUW There were 70 in both groups.

LEVER The other possibility to consider, of course, is that it may not just be the age of the patient, it may be the age of the erythrocyte. A rapidly turning over population has a high proportion of young eager pumping erythrocytes which might have a rather different result than a population of rather subdued exhausted old erythrocytes. So survival of red cells may be important rather than any fundamental difference between cell transport in the two diseases.

de ZEEUW I agree with you, I think that should also be checked but that doesn't explain that there is a diurnal variation.
LEVER Have all your hypertensives never been treated?

de ZEEUW Yes they were treated before but they were on at least four weeks of placebo therapy.

LEVER How do you know that hasn’t affected them?

de ZEEUW This was in a multicentre study which we did with enalapril and so we had these regular checks every two weeks.

BIANCHI (Pisa) You put our group together with the people who found a lower co-transport, this is not correct because we observed that patients could have either a lower or higher co-transport compared to controls. The final results could well be that if you compare the averages of the controls and hypertensives you might come out with the similar values. Firstly can you give us some more detail about the technique you use? You mention that you overload the cells for 20 hours and then you measure the sodium efflux, but with what methods and what conditions?

de ZEEUW As you are probably aware if I gave a talk about the conditions in which I did the study I would be here for the next twenty minutes. I will suffice to say we use the same technique as Garay* described in his paper about measuring co-transport and we specially did this because the Paris group was one of the first groups to show this distinct difference between essential and secondary hypertension. We are well aware of the fact that after that a number of different techniques have been used, especially with respect to the loading of the erythrocytes for instance the group of Garraham and Rega† load their erythrocytes to about 60mmol internal sodium. This could well be the explanation for the fact that they find an increase instead of a decrease in sodium efflux.

BIANCHI Yes, I think this is very important for two reasons. First, are you sure that the variation during the day was mainly on sodium efflux while the potassium efflux was the same? By definition this is not co-transport but is something that selectively affects sodium but not potassium. The second point is you may have a 300 per cent change with menstrual cycle, and this should be taken into account in your interassay variations.

de ZEEUW I don’t agree with you. I think that this even stresses the fact that measuring the co-transport in two populations without any restrictions is a bad thing to do. The six individuals, the normotensive volunteers, in which I measured the day-to-day variation in the urine were all male, in fact one of them was me.

SALTISI (London) I wonder with the recognition of parathyroid hormone as a Na⁺-K⁺ ATPase inhibitor have you looked at the diurnal variation of Na⁺-K⁺

*Garay RP, Meyer Ph. Lancet 1979; i: 349
co-transport in relation to parathyroid hormone or other diurnally varying hormones, particularly cortisol?

de ZEEUW We did not look at PTH but we did look at renin as was also published by Edmundson* and he found that renin was related to the ouabain sensitive sodium efflux. We measured renin in these subjects but we didn’t find correlation with that.