Erythrocyte cation transport and hypertension
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SUMMARY AND CONCLUSIONS

This thesis deals with cation transport over the cell membrane of the human erythrocyte. The results of investigations on the technique of measurement of cation transport and its application in studies on erythrocyte sodium metabolism are described. In addition, we report on a clinical study and a literature survey concerning the relation between cation transport and essential hypertension.

As early as in 1952, Tobian and Binion suspected a relation between a defective intracellular sodium metabolism and the pathogenesis of essential hypertension. They observed increased sodium concentrations in the vascular wall of renal arteries in patients with essential hypertension in comparison with normotensive controls. Gradually, the interest in intracellular sodium metabolism shifted to erythrocytes of hypertensive patients since Losse observed an increased erythrocyte sodium content as well in 1960. In addition, the detailed knowledge of the metabolism of the erythrocyte and its easy isolation stimulated the use of this type of cell for cation transport studies in relation to hypertension.

The sodium and potassium concentrations in the erythrocyte are maintained at constant levels by the concerted action of Na⁺(K⁺) transport systems located in the cell membrane. The ouabain-sensitive Na⁺/K⁺ transport (Na⁺/K⁺ pump), the furosemide (lasix)-sensitive Na⁺/K⁺ cotransport and the phloretine-inhibitable Na⁺/Li⁺ counter-transport can be distinguished.

In 1979, the group of Garay published a report which drew the attention of workers in the field of hypertension. The authors claimed a complete discrimination between normotensive and essential hypertensive individuals by means of a non-invasive laboratory test based on Na⁺/K⁺ cotransport measurement in erythrocytes. However, the application of their methods by other investigators led to conflicting results, possibly in part as a result of variability in transport assays and the (clinical) characteristics of the populations under study.

Usually, cation transport in erythrocytes is measured by incubating the cells in artificial media after several preparatory steps like washing, centrifugation, incubation, etc. The conditions of incubation may vary in design and performance (i.e. composition...
of media, incubation time) between the different investigators. Besides, the mainly manual procedures are laborious and time-consuming. They allow processing of only small numbers of samples of relatively large volume. This urged us to develop a \(^{22}\text{Na}\) tracer method for the measurement of the sodium efflux rate constant \(k_e\) (i.e., the fraction of intracellular sodium exchanged for extra-cellular sodium per hour). The method, described in Chapter 2, offers high sample capacity, rapidity and reliability which was achieved by miniaturization and standardization of hitherto manual procedures. In short, washed erythrocytes obtained from venous blood are "loaded" with tracer amounts of \(^{22}\text{Na}\). The "loaded" cells are suspended in plasma or artificial medium by means of especially designed and constructed apparatus. After incubation for 5, 20, 35 and 50 minutes the cells and medium are separated. Both are assayed for radioactivity in a scintillation counter. From the changes with time of the measured radioactivities, the flux rate constant, \(k_e\) can be calculated. The method allows the measurement of 48 \(k_e\) values per day, using only 0.75 ml of blood per \(k_e\) value.

Most cation transport studies have been carried out by incubation of the erythrocytes in artificial medium instead of their natural environment: plasma. A drawback of this choice, however, is uncertainty about the correct composition of the media. Therefore, we studied the effect on \(k_e\) of replacement of plasma by two current artificial media of different composition. Secondly, we investigated the cause of differences in \(k_e\) values measured in plasma and artificial medium (Chapter 3). Both artificial media "Balanced Salt Solution" (BSS) and Hanks' solution (Hanks') contained the same sodium and potassium concentration but differed in concentrations of a number of other solutes. It appeared that both total and ouabain-sensitive \(k_e\) were lower in Hanks' (10%) and BSS (25%) as compared with plasma.

In the search for the cause of the observed differences we carried out dialysis experiments. After dialysis of plasma against Hanks' over a membrane with relative molecular mass cut-off of 1000 Da, the \(k_e\) in plasma decreased to the level as measured in Hanks'. Thus, it seemed likely that dialyzable sodium transport stimulating factors are present in plasma. Dilution experiments of plasma with Hanks' revealed that the factors are present in excess.

In Chapter 4, we describe the further characterization of the factor and the search for its mechanism of action. Dialysis of plasma against artificial medium using membra-
s. Besides, the mainly low processing of only led us to develop a \(^{22}\)Na constant \(k_r\) (i.e. the fraction hour). The method, and reliability which was usual procedures. In short, with tracer amounts of medium by means of for 5, 20, 35 and 50 for radioactivity in a and radioactivities, the flux measurement of \(48k_r\) values

The measurement of the erythrocytes. A drawback of this medium. Therefore, current artificial media of differences in \(k_r\) values by means of artificial media "Balanced the same sodium and number of other solutes. It Hanks' (10%) and BSS (10%) and we carried out dialysis membrane with relative to the level as measured the factor(s) are revealed that the factors the factor and the search medium using membra-nes with varying molecular cut-off points revealed relative molecular mass(es) of the factor(s) of 100-1000 Da. The factor(s) could be absorbed on Dowex at pH 1.5 and Amberlite at pH 11.0, indicating "Zwitterionic" character. They are hydrophylic and resistant to acid hydrolysis. These characteristics and direct measurements of contents made amino acids likely candidates for the efflux stimulating properties of the factor(s). Indeed, plasma amino acids added to artificial medium could abolish the sodium efflux difference between plasma and the artificial medium. To test whether the factor(s) act via a mechanism whereby inactive, existing Na'K pump sites are directly "unmasked", we measured the number of ouabain binding sites per erythrocyte in the presence and absence of the factor(s). This number did not differ between both conditions, indicating no direct action of the factor(s) on the Na'K' pump. We speculated whether the amino acid-induced sodium efflux stimulation was secondary to an increase of sodium influx. Therefore, the influx rate constant, \(k_i\) in plasma, Hanks', Hanks' with added isolated factor(s) and Hanks' containing a plasma profile of amino acids was measured. Significantly higher \(k_i\) values in plasma versus Hanks' were found. However, no effects on \(k_i\) were observed using isolated factors or plasma amino acids. Apparently, plasma contains sodium influx stimulating factors which are not amino acids. Equilibrium dialysis of plasma against Hanks' demonstrated that these factors are dialyzable as well and have relative molecular mass(es) below 1000 Da.

As mentioned above, erythrocyte sodium transport measurement for diagnostic purposes carried out by various investigators led to conflicting results. We investigated one of the clinical parameters that could possibly account for the discrepancies. In Chapter 5 we describe the result of a clinical study in which Na'/K' cotransport fluxes across the red cell membrane were determined in 38 normotensive volunteers and 33 patients with established essential hypertension. No differences were found in flux values between these two groups. For 22 patients the effect of antihypertensive medication on the cotransport was followed. It appeared that neither propranolol \((n = 11)\) nor enalapril \((n = 11)\) had any effect on the transport. In addition, it appeared that individual diurnal and day-to-day variation of the fluxes are substantial.

In Chapter 6 we present the results of a meta-analysis of 17 years of literature on Na'/Li' countertransport (NLCT) and Na'/K' cotransport (COT) measurement in relation to essential hypertension. The analysis was aimed at answering two main questions:
1. Which clinical or laboratory variables influence NLCT and COT flux values in Caucasian populations? 2. How useful are NLCT and COT measurements as a diagnostic aid in essential hypertension? We found that essential hypertension, family history for hypertension, gender and antihypertensive medication are main determinants for the flux values of both transport systems. Even taking into account these determinants, large standard deviations of the weighed mean flux values of the integrated studies were calculated, however. This suggests the existence of other, unknown, variables affecting the flux rates. Significant differences in weighed mean flux values between sub-groups of normotensives and hypertensives could be demonstrated. However, these differences are much smaller than the variance in the weighed mean flux values. Therefore, NLCT and COT measurement cannot be of diagnostic use in essential hypertension.

Conclusions:
Erythrocyte cation transport measurements did not lead (unlike the early expectations) to the hoped-for result: a diagnostic tool for essential hypertension.

Our meta-analysis demonstrated the cause of that failure. Known and unknown clinical variables influence the cation transport measurement to the extent that there exists a too large overlap between normotensive and essential hypertensive populations.

Cation transport measurements are useful however, in within-person studies in order to reveal the effect of changes in clinical variables on a transport system for example. As our studies demonstrated one must keep in mind that the composition of the media in which the erythrocytes are incubated may influence the activity of the transport system under study. Measurements in artificial media may not give reliable information on the in vivo (plasma) activity of the erythrocyte transport system in the erythrocyte membrane. In this respect systematic investigations on the effects of isolated and defined plasma factors added to artificial media are required to obtain their optimal composition.

The large variabilities in clinical and laboratory methods thus far observed in the cation transport measurements illustrate the need for international standardization of the methods.