Particularities of Sheep Erythrocyte Membrane and Mineral Centents

Tongyai S.[1] Al-Kotobe M.[2]

1. Faculty of Veterinary Medicine, Kasetsart University, THAILAND

2. Laboratoire des Maladies Metaboliques, INRA, Theix, FRANCE

Abstract

The presence of HK and LK type erythrocyte was confirmed in the experimental cross-bred sheep herd. The relationship between the highly different levels of erythrocyte potassium in the two groups and the levels of intracellular magnesium was put forward. High potassium (HK) erythrocytes also contain significantly higher magnesium level than the low potassium (LK) type cells while intracellular sodium was inversely related to potassium level. Fluorescence polarization studies with the use of the lipid probe, diphenylhexatriene (DPH), showed a slightly higher membrane fluidity of the LK erythrocytes than that of the HK type cells ($P < 0.05$). The results, thus, suggested that the characteristic of LK erythrocyte is likely to result from a limited active Na-K pump activity for inward K transport due to the low Mg level and a more permeable membrane for passive ionic movement along the concentration gradient than that of the HK type cells. Therefore, K entry could not meet the higher outward movement.

บทคัดย่อ

เม็ดเลือดแดงของแกะทดลองลูกผสมประเภทเดียวกันแยกกลุ่มได้เป็น พวกที่มีโปแตสเซียมภายในเซลล์สูงและพวกที่มีโปแตสเซียมภายในเซลล์ต่ำอย่างมีนัยสำคัญ ($P < 0.001$) ระดับโปแตสเซียมมีความสัมพันธ์กับระดับแมกนีเซียมภายในเซลล์โดยที่พวกที่มีโปแตสเซียมภายในเซลล์สูงจะมีแมกนีเซียมสูงและพวกที่มีโปแตสเซียมภายในเซลล์ต่ำจะมีแมกนีเซียมต่ำร่วมด้วย ส่วนระดับโซเดียมภายในเซลล์นั้นแปรผกผันกับระดับโปแตสเซียม การศึกษาคุณสมบัติเคมี-ฟิสิกส์ของผนังเซลล์เม็ดเลือดแดงด้วยเทคนิกฟลูโอเรสเซนส์โพลาไรเซชั่นโดยใช้สารลิปิดเรืองแสงไดเฟนิลเฮกสะไทรอีน แสดงผลถึงการที่เซลล์เม็ดเลือดแดงแกะชนิดที่มีโปแตสเซียมต่ำมีผนังเซลล์ที่อ่อนเหลวกว่าเซลล์ชนิดที่มีโปแตสเซียมสูง ($P < 0.05$) ผลการศึกษาทั้งหมดบ่งว่าลักษณะการที่เซลล์ที่มีโปแตสเซียมต่ำอาจเป็นผลเนื่องมาจาก การที่ปั๊มโซเดียม-โปแตสเซียมมีขอบเขตการทำงานต่ำในการลำเลียงโปแตสเซียมกลับเข้าสู่เซลล์ร่วมกับการที่ผนังเซลล์มีคุณสมบัติยอมให้ไอออ้น เช่น โปแตสเซียมและโซเดียมเคลื่อนผ่านในทิศทางตามความแตกต่างของความเข้มข้นระหว่างภายในและภายนอกเซลล์โดยไม่ต้องใช้พลังงานได้ดีกว่าผนังเซลล์ที่มีโปแตสเซียมสูง

Introduction

As early as 1898, Aberhalden drew attention to the fact that sheep erythrocytes could have low intracellular potassium. It was not until 1954 when Evans demonstrated clearly that there exists two distinct red blood cell types in sheep, the high potassium or HK type containing 80 - 90 mmoles/1 cells of potassium and the low potassium or LK type containing 20 - 30 mmoles/1 cells of potassium. However, plasma levels of potassium and sodium are not different between these two types. Such LK/HK polymorphism can be found in nearly all breeds of sheep and has been shown to be genetically controlled with the genes for LK type behaving dominantly to those for HK.

It is often observed that intracellular potassium and magnesium content behave in a relative fashion such as in the case of magnesium deficiency where decrease intracellular Mg is often accompanied by loss of cellular K (Franscisco et al., 1981; Heaton et al, 1987). Moreover, membrane permeability is closely related to membrane physico-chemical properties. It is, therefore, of our interest to study certain characteristics of erythrocyte mineral status and the physicochemical properties of sheep red cell membrane under normal condition, of which, to our knowledge, have not been shown elsewhere.

Materials and Methods

Animals and Feed

The studies were conducted in non-pregnant Limousine : Romanov cross-bred ewes aged 2 - 5 years. The average weight of the animals were 55 $\pm$ 1 kg. The ewes were given hay and water ad libitum. Protein level of the hay was 9.5% DM and crude cellulose level was 34% DM.

Blood Collection

Blood samples were taken from the jugular vein into heparinized tubes and immediately centrifuged at 1300 g for 15 minutes at 4° C. Plasma was separated and kept at -20° C for further investigation. The pack red cells were used for membrane preparation and intracellular mineral studies.

Membrane Preparation

Erythrocyte membranes were prepared according to the method of Hanahan and Ekholm (1974) by washing the pack red cells twice in isotonic Tris-HC1 buffer, pH 7.6, then lysing and washing 3 times in hypotonic Tris-HC1 buffer, pH 7.6 at 4° C. The suspension of erythrocyte membranes in the final medium corresponded to approximately 6×10^a red cells/ml.

Fluorescence Polarization Measurements

An aliquot of membrane suspension was incubated for 1 hour at 24° C in freshly prepared dispersion of the fluorescence probe, 1, 6 - diphenylhexatriene (DPH) in 20 mOsM Tris-HC1, pH 7.6. The labelled membranes were immediately measured for fluorescence with a spectrofluorimeter equipped with a polarizer unit at constant temperature of 24° C. The measured values of parallel and perpendicular fluorescence intensities in relation to the polarized excitation beam depended on the motion of the probe according to the bulk fluidity of the membrane and were used in the calculation of membrane microviscosity according to Perrin's equation (Shinitzky and Barenholz, 1978). The microviscosity parameter, expressed in poise, is inversely proportional to the fluidity of the membrane.

Mineral Analysis

Mineral concentrations in the plasma (Na, K, Mg, Ca) and washed red cell hemolysate (Na, K, Mg) were determined by atomic absorption spectrophotometry following the recommendations of PERKIN ELMER (1973). Plasma inorganic phosphate content was analysed by modified technic of Taussky and Shorr (1963).

Result and Discussion

Ninety four sheep in the experimental herd were studied in relation to the characteristic HK, LK type erythrocytes. Plasma mineral content of the herd was in the normal zone for sheep and no significant variations were observed. The values were, therefore, demonstrated in Table 1 as the mean of all 94 sheep studied. Thirty nine percent of the studied herd was found to be HK type characterized by a 5-fold higher intracellular potassium concentration ($P < 0.001$) and a 4-fold lower intracellular sodium concentration than that of the LK group ($P < 0.001$; Table 1). The results thus confirmed the highly different levels of erythrocyte K and Na in LK and HK type sheep shown by Evans (1954).

New evidence of a relationship between the LK and HK characteristics and the level of intracellular Mg was additionally put forward for the first time. Erythrocyte magnesium presented a significant difference between the HK and LK groups, being higher in the HK than in the LK group ($P < 0.001$, Table 1). It has been shown that erythrocyte and plasma Mg concentration are closely controlled by genetic factors in mice as well as in man originated from various zones of the world. The difference persisted in spite of the changes in actual residential climates (Henrotte, 1981; 1982). Taking this evidence into account, it is likely that intracellular Mg would also be genetically controlled in ovines which could explain the polymorphism observed in the HK and LK groups.

Table 1 Plasma and erythrocyte mineral content in the experimental sheep

	K (mM)	Na (mM)	Mg (mM)	Ca (mM)	P (mM)
Plasma (94)	4.88 ± 0.07	149 ± 1	0.93 ± 0.03	2.38 ± 0.03	1.94 ± 0.12
Erythrocytes					
- HK (37)	88 ± 1	21 ± 1	1.32 ± 0.02		
- LK (57)	16 ± 2***	89 ± 1***	1.20 ± 0.01***		

Mean Values ± SEM, values in parentheses are numbers of animals studies, Significant difference between the LK and HK type cells ***$P < 0.001$

Na and K can be transported across red cell membrane by various mechanisms, principally, the passive diffusion along electrochemical gradient, the active Na pump and chloride dependant - Na, K cotransport. Other minor pathways include exchange related to lithium or antiport exchange with calcium. Na can also be transported coupled to amino acids. However, the most important mechanisms of mineral transport are those controlled by ATPase activity (Na-K ATPase, Mg-Ca ATPase). The factors which intervene in the regulation of these enzyme activities could be metabolic, hormonal or genetic.

Na pump in red cells regulates intracellular volume and ion composition by exchanging intracellular Na^+ for extracellular K^+ usually at a $3Na^+ : 2K^+$ stoichiometry. The mechanism is accompanied by ATP hydrolysis engaging the activity of $Na^+ - K^+$ ATPase. Mg is an important co-factor in the ATPase activity and the ATP utilization for the transport function. The result of sheep erythrocyte Mg level suggested that the LK type red cells could possess limited Na-K pump activity due to lower Mg activation of the ATPase system than that of the HK type cells rather than a simple difference in the number of pump units present.

A close relationship of intracellular Mg and K levels has also been shown during Mg deficiency. Tissue cells tend to lose both cellular Mg and K while Na and Ca tend to accumulate within the cells (Francisco et al., 1981; Heaton et al., 1987). Recent demonstration of membrane modification during magnesium deficiency could explain the imbalances (Tongyai et al., 1988; abstract). The dynamic state of membrane lipid and protein is an indicator of the possibilities of ionic leakage through membrane channels or pores along the concentration gradient. For this reason, the fluorescence polarization technique was used to assess the fluidity of erythrocyte ghosts.

The hydrophobic probe DPH which can penetrate both the solid and liquid phases of the lipid bilayered at a given time, provides an index of molecular order in the lipid region of the membrane. As shown in Table 2, there exists a significant difference in the membrane fluidity of the HK and LK type red cells measured in an inversely related value as microviscosity ($P < 0.05$). With the LK membrane being more fluid than that of the HK type cells, our result indicated a more dynamic and permeable membrane of the LK type cells to passive ionic fluxes.

Table 2 Membrane fluidity studies of LK and HK type erythrocytes measured by fluorescence polarization technique

Probe	Parameter	LK	HK	P-value
DPH	microviscosity (poise)	12.26 ± 0.37	13.83 ± 0.49	< 0.05

Mean Values ± SEM of 20 sheep per group

Membrane lipid and protein composition and subsequently the membrane fluidity is under the influence of several factors, namely, genetics, nutritional status and acclimatization. The measurement of membrane microviscosity in the order of 12 - 14 poise indicated that sheep red cell membrane is highly rigid compared to that of other species. This is due to the fact that sheep red cell membrane contained very high level of rigidifying lipid, the sphingomyelin. In our experimental condition, it is likely that the difference in red cell membrane physicochemical properties is under genetic influence. Further investigation of membrane composition is required in terms of the level of membrane fluidifiers and rigidifiers.

Conclusion

The results of the studies showed new evidence of the difference in intracellular Mg level and membrane fluidity between the sheep LK and HK type red cells. The lower Mg content and the higher membrane fluidity of the LK type red cells compared to the HK type cells suggested a limited Na pump activity and a high passive membrane ionic permeability, respectively. Such combined effect could result in greater passive loss of intracellular K than the amount actively returned inward, thus, a subsequent characteristically low intracellular K and a high Na content of the LK cell type could be observed.

Acknowledgement

The authors would like to thank Dr. Y. Rayssiguier and Dr. C. Motta for their advice and the use of the scientific equipment in their laboratories for these studies.

References

EVANS, J.V. (1954) Electrolyte concentration in red blood cells of British breed of sheep. Nature, London, 174, 931 - 933.

FRANCISCO, L.L., SAWIN, L.L., DIBONA, G.F. (1981) Mechanism of negative potassium balance in the magnesium-deficient rat. Proc. Soc. Exp. Biol. Med., 168, 382 - 388.

HANAHAN, D.J, AND EKHOLM, J.E. (1974) Methods in enzymology edited by Fleischer S. and Pacher L., Academic Press, New York, 31, part. A. p. 168 - 172.

HEATON, F.W., TONGYAI, S., MOTTA, C., RAYSSIGUIER, Y. and GUEUX, E. (198) Changes in the erythrocyte membrane during magnesium deficiency. Nutr. Res. 7 : 655 - 663.

HENROTTE, J.G. (1981) Facteurs des regulations du metabolisme magnesique chez l'hommme. Magnesium Bull., 3, 237 - 248.

HENROTTE, J.G. (1982) Genetic regulation of red blood cell magnesium content and major histocompatibility complex. Mini review. Magnesium, 1, 69 - 80.

PERKIN ELMER "Cookbook", (1973) Analysis of serum determination of Ca, Mg, Na, K. Page ref. BCI-R. Perkin Elmer Corp. Inc., Norwalk, Connecticut

SHINITZKY, M. AND BARENHOLZ, Y. (1978) Fluidity parameters of lipid regions determined by fluorescence polarization. B.B.A., 515, 367-394.

TAUSSKY, H.H. AND SHORR, E. (1963) A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem., 202, 675 - 685.

TONGYAI, S., RAYSSIGUIER, Y., MOTTA, C. (1988) Modification of erythrocyte membrane during magnesium deficiency. Abstract. The 26th Kasetsart University Scientific Conference, 3 - 5 February, 1988, Kasetsart University, Bangkok, Thailand.