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CHANGES IN K^+ PUMP TRANSPORT AND OUABAIN BINDING SITES IN ERYTHROCYTES OF GENETICALLY LOW K^+ LAMBS

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Summary

Red cells from 3 genetically low K^+ lambs exhibited: high cell K^+ and K^+ pump activity and 135 pumps/cell on day 3 after birth; increased cellular Na^+, and a decrease of the number of pumps relative to K^+ pump flux on day 17; and low cell K^+, low K^+ pump flux and about 40—50 pumps/cell on day 40 or later.

At birth, all lambs possess high potassium (HK) red blood cells. However, during the first 40—60 days, the cation composition of red cells from lambs endowed with the dominant low potassium (LK) gene [1,2] gradually changes to LK, high sodium steady state levels, while the HK character of lambs with the recessive HK gene remains unaltered throughout life [3—5]. The 'HK-LK transition' observed in red cells of LK lambs was initially attributed to cellular maturation and/or replacement [5,6], but recently we showed that cellular replacement appears to determine the conversion from the HK to the LK state since 3 erythrocyte volume populations were distinguished [7].

Adult LK sheep red cells have fewer Na^+K^+ pumps which are kinetically different [8] as compared to adult HK cells [9]. Furthermore, the HK-LK dimorphism is genetically associated with two distinct membrane antigens: homozygous LK cells contain only the L antigen [10,11], and HK cells only the M antigen [12,13], while heterozygous LK cells possess both L and M antigens. About 1/3 to 1/2 of the 900—1500 L antigens/cell [14,15] are functionally associated with the cation pumps (Lp-antigens, refs. 16—19) as evident from the effect of anti-L, an antibody modifying the kinetic properties of the LK pump [20].

In terms of a genetic and functional explanation of the HK-LK transition in lambs, it is important to consider simultaneously the temporal change

in cellular cations, K^+ pump transport and the number of pumps per cell, and to correlate these parameters to the appearance of the stimulatory anti-L effect. Earlier we reported that anti-L did not stimulate the K^+ pump in red cells of new born LK cells but did so in red cells of young sheep beyond 40 days of age [7]. Here we demonstrate that the high K^+ pump activity found in the early, HK-like red cells of 3 newborn LK lambs was paralleled by a high number of ouabain binding sites and, therefore, pumps per cell. During the first 6—8 weeks after birth, the number of pumps was gradually reduced and a change of their kinetic properties appeared.

The lambs used in this study were heterozygous (LM) Dorsets, the product of homozygous (LL) LK ewes and an HK (homozygous MM) ram; animals J1 and J2 were twins. For each time interval, 5 ml of blood were drawn by venipuncture into heparinized syringes. Hematocrit, mean corpuscular hemoglobin, and mean cell volumes were determined on whole blood by standard techniques [9,21].

Cells were washed 4 times at 4° C in buffer containing (mM): 135 NaCl/5 KCl/10 sucrose/10 glucose/10 Tris·HCl, pH 7.4 at 37° C. Suspensions were adjusted to 10% hematocrit for use. After warming to 37° C, [^{42}K]- and [^{3}H]-ouabain ($1—4 \cdot 10^{-7}$ M final concentration) were added to appropriate suspensions, and sampling proceeded over 60—90 min. Duplicate samples were assayed for cations, [^{42}K]- and [^{3}H] ouabain using hemoglobin determinations (absorbance at 527 nm) to estimate the number of cells in each sample. The details of these methods and the calculation of pump flux and bound ouabain have been published elsewhere [9,21,22]. Since [^{42}K] uptake and [^{3}H] ouabain binding were assayed on the same cell samples, the amount of bound cardiac glycoside could be correlated with the degree of K^+ pump inhibition obtained. The reported number of ouabain molecules per cell (Fig. 1C) represents an extrapolation of the ouabain binding data to 100% K^+ pump inhibition.

Since significant changes occurred during the maturation periods in cellular volume as well as hemoglobin content and concentration [7], the data for the cation content and fluxes and for ouabain binding were expressed in units pertinent to each parameter. Intracellular cation compositions were reported as mmol/l cell water; the volume fraction of water was estimated from the hemoglobin content, cell volume, and hemoglobin density. K^+ pump flux was referred to a fixed number of cells (mmol $K^+/10^{13}$ cells per h). Although this unit may conceal differences in fluxes due to surface area changes in the maturing cell populations, it avoids artifacts arising from different cell volumes and hemoglobin concentrations and contents. Ouabain binding was expressed in molecules per cell.

On the basis of our previous study [7] 4 particular days were chosen for bleeding on which erythrocyte populations I (day 3), II (day 17), and III (days 36—44, and 121—129) were likely to be present in the peripheral blood. Fig. 1 compares the changes observed in intracellular cations (A), K^+ pump fluxes (B), and ouabain binding at 100% K^+ pump inhibition (C). Red cells of 3-day old lambs were of HK type (140—170 mM K^+, and 10 mM Na^+ per l cell water) with a K^+ pump flux in the range of or slightly higher than normally found in adult HK cells [23]. The number of ouabain binding sites was about

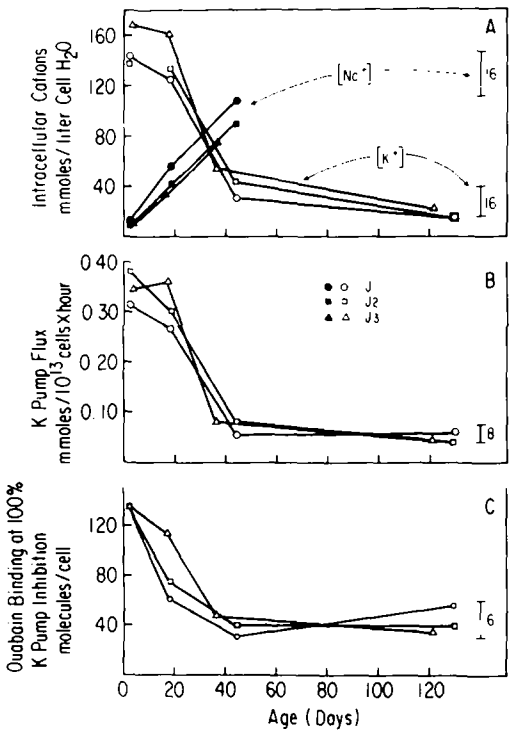

Fig. 1. Change of intracellular cation concentrations (A), K^+ pump flux (B), and ouabain binding sites (C) in red cells of 3 lambs at four different bleeding dates after birth. Bars indicate the range of each parameter found in LK cells of different adult sheep (number/bar).

135/cell and thus close to values reported for adult HK cells [9]. The turnover numbers (K^+/site per min) were between 2300 and 2800, also in the range of values found in adult HK cells. Hence, the activity of the Na^+K^+ pumps in these cells, belonging almost entirely to the neonatal cell population I [7], resembled adult HK pumps.

Red cells obtained on day 17 may be still classified as HK cells (125—160 mM K^+/l cell water); however, the cellular Na^+ concentration, $(Na^+)_c$, rose to 30—55 mM/l cell water, indicating an increment in the total cation concentration not readily explained at present. Furthermore, the mean K^+ pump activity in the red cells of the twin lambs J1 and J2 was lowered by 20% while the number of ouabain binding sites was reduced by 50%. As compared to day 3 cells, K^+ pump flux and ouabain binding sites in red cells of lamb J3 changed much less; also, the red cells of this lamb had the highest $(K^+)_c$ on day 3. The mean turnover numbers for the pumps of the twin lambs were 4200 and thus about 60% higher, and those for cells of lamb J3 about 24% higher than found in red cells of day 3 lambs. This slight discrepancy may reflect individual differences between the twins and lamb J3. The generally higher turnover numbers, however, suggest stimulation of the existing pumps by the elevated $(Na^+)_c$, since intracellular Na^+ activates K^+ pump flux [8,24]. This conclusion implies a change in the number rather than the properties of the existing Na^+K^+ pumps in red cells of 17-day old lambs.

In red cells from days 36 (lamb J3) and 44 (twins) the number of ouabain binding sites/cell declined further, and K^+ pump flux sharply decreased to levels indistinguishable from those measured in red cells of 121- and 129-day old lambs or in red cells of 8 adult LK controls (Fig. 1B, bar). Thus, around day 40, the turnover numbers were about 2000 and 1700 for the red cells of the twins and lamb J3, respectively, and further decreased to 1000—1200 as $(Na^+)_c$ rose to 80—110 mM/l cell water at the expense of $(K^+)_c$. Hence, the red cells studied around day 40 and thereafter exhibited properties typical for adult LK red cells: 40—60 pumps/cell; LK-type turnover numbers, and, relative to HK cells, the inability of increased $(Na^+)_c$ to offset the inhibitory action of K^+ at the Na^+ loading site of the pump [20].

The transport changes observed during the HK-LK transition in red cells of new born LK lambs of this study are corroborated by several other reports: The Na^+ (and $Na^+ + K^+$)-ATPase activity of LK lamb red cells fell during the first 40 days from HK to LK levels [6,25], whereby the HK type kinetics persisted [25]. K^+ pump influx [5,6] and the parameter β, the pump to leak ratio, which is high at birth, fell progressively from birth to six weeks of age to low values typical for LK cells [6]. There is a report of 33, 36, and 29 ouabain binding sites/cell in 11-, 14-, and 19-day old lambs, respectively [6].

The quantitative (reduction of pump numbers) and qualitative (increased inhibition of the pump by cellular K^+) changes must be considered in light of the association of the Na^+K^+ pump in HK and LK sheep red cells with the M and L antigens, respectively. These antigens are minimally expressed on red cells of new born lambs and gradually appear as the animal matures [7,15,27,28]. Extending our earlier findings [7], it is inviting to reason that the lamb of LK type produces three consecutive red cell populations, each with fewer HK pumps than its predecessor and that, upon full development of the L antigens, kinetic modification of these pumps occurs causing their inhibition by cellular K^+. Only when the L antigen is fully expressed, and functionally (and perhaps structurally) linked up with the Na^+K^+ pump, anti-L will reduce the inhibitory action of the L antigen. However, other possibilities cannot be ruled out at present to explain the HK-LK transition in genetically LK lambs.

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