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THE NUMBER OF Na^+:K^+ PUMP SITES ON RED BLOOD CELLS FROM HK AND LK LAMBS

PHILIP B. DUNHAM* AND JOSEPH F. HOFFMAN

Department of Physiology, Yale University School of Medicine, New Haven, Conn. 06510 (U.S.A.)

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SUMMARY

Lambs of known genotype with respect to the locus determining cation composition of red cells were obtained by selective matings. Numbers of K^+ pump sites per cell were determined on HK and LK lambs 10–20 days postnatal by simultaneously determining [^{3}H]ouabain binding and inhibition of active K^+ transport. Red cells from HK lambs were indistinguishable from adult HK cells with regard to the K^+ pump flux and number of pump sites. Cells from genetically LK lambs had pump fluxes and numbers of pump sites intermediate between those from adult HK and LK sheep. The results suggest that the change in cation composition and decrease in the K^+ pump during the first 60 days in genetically LK lambs can be correlated with a reduced number of K^+ pump sites.

Populations of sheep are dimorphic with respect to the cation concentrations in their red cells. High-potassium (HK) individuals have 70–90 mM K^+ per l cells and 10–20 mM Na^+ per l cells, while the red cells in low-potassium (LK) individuals have approximately the converse concentrations of Na^+ and K^+ (refs. 1 and 2). These phenotypes are controlled by a single genetic locus with two alleles, the allele for LK being dominant[3,4]. Both cell types have a coupled active transport system for K^+ and Na^+, but the rate of transport under physiological conditions is 4–8 times greater in HK cells[5,6]. In addition, LK cells are 4 times more permeable to K^+, and are probably more permeable to Na^+ as well. The difference in transport rates can be explained by a difference in number of transport sites, measured by simultaneous determinations of [^{3}H]ouabain binding and inhibition of K^+ transport[6]. The number of pump sites (ouabain binding sites) per cell are 42 for HK and 7.6 for LK cells.

In genetically LK sheep, red cells newly released into circulation have cation concentrations and transport characteristics like HK cells. Several weeks are required for maturation into cells with the characteristic LK phenotype, as shown by studies on circulating reticulocytes in adult sheep[7]. A similar phenomenon occurs in LK lambs; in prenatal and immediately postnatal lambs known from their parentage to be LK, the red cells are like cells from HK sheep with regard to cation composition

* Permanent address: Department of Biology, Syracuse University, Syracuse, N.Y. 13210, U.S.A.

and transport. During about 60 days there is conversion to the LK phenotype, but replacement of fetal by adult cells may be the predominant mechanism[8–11]. In HK sheep, there is no evidence for these kinds of changes in the red cells in lambs or in adults.

A hypothesis has been proposed in an attempt to explain these genetic, developmental, and physiological aspects of the HK–LK system. Assuming that both pump and passive, or leak, K^+ fluxes occur through specific sites in the membrane, it was postulated that red cells in HK and LK sheep have the same total number of cation transfer sites[6]. In HK cells most of the sites are pump sites and in LK cells most are passive or leak sites. The genetic locus determines the fractions of the sites which are pumps and leaks. In both LK and HK sheep newly released red cells are phenotypically HK. The LK allele causes a lability of a majority of the transport sites which results in their conversion from pumps to leaks. Recent evidence indicates that treatment of LK cells with antiserum prepared against other LK cells causes a dramatic increase in the K^+ pump flux[12]. It has also been shown that the antiserum causes an increase in the number of ouabain binding sites per cell[13]. In the terms of the above hypothesis the antibody could act by converting leak sites back to pumps. Consistent with this interpretation are preliminary data which indicate that a decrease in leak flux accompanies the increase in the pump caused by the antibody[13]. Furthermore, a recent preliminary report indicated that reticulocytes from LK sheep have a relatively high number of ouabain binding sites[14].

The present experiments were undertaken to determine if a decrease in the number of pump sites per cell accompanied the other changes which occur in the red cells of genetically LK lambs. Adult rams and ewes were selected from a flock of Dorsets maintained near New Haven by the Division of Animal Sciences, Yale University School of Medicine. The genotypes were determined from the phenotypes with respect to cation composition and the antigenic factor M (*cf.* ref. 6). The genes for these two characters are closely linked; the dominant allele M-positive is linked with the recessive allele for HK, and the recessive allele M-negative is linked with the dominant LK allele[15]. By selective breeding lambs of known genotype were obtained. At age 10–20 days the lambs were anaesthetized with nembutol and exsanguinated from the femoral artery, facilitated by simultaneous infusion with heparinized saline *via* the femoral vein.

The red cells were washed immediately by centrifugation in the cold. This procedure, as well as the methods for simultaneous determinations of [^{3}H]ouabain binding and K^+ transport were carried out exactly as described previously[6]. In brief, the cells were equilibrated at 37° up to 2 h in 0.01–0.1 μM [^{3}H]ouabain, washed again in the cold, and divided into two portions. From one, [^{3}H]ouabain was extracted for scintillation counting. The other portion was returned to 37° for determination of K^+ influx using $^{42}K^+$. From fluxes determined on cells not exposed to ouabain and others exposed to a high (0.1 mM) ouabain concentration, the K^+ pump flux and the percent inhibition caused by the bound [^{3}H]ouabain were determined. Ouabain molecules bound per cell, percent inhibition of the K^+ pump, and number of pump sites per cell (ouabain molecules at 100% inhibition) were calculated as before[6]. The specific activity of [^{3}H]ouabain used here in calculating total ouabain sites per cell is the same as that used in ref. 6, and is subject to the same uncertainty as discussed in that paper.

TABLE I

CATION CONCENTRATIONS, K^+ FLUXES, AND PUMP SITES IN HK AND LK RED CELLS OF YOUNG AND ADULT SHEEP

Age given in days; $[K^+]_i$ and $[Na^+]_i$ represent intracellular concentrations in mM/l cells; i_{MK}^P and i_{MK}^L represent pump and leak influxes of K^+, respectively, in mM/l cells per h; total sites means the number of ouabain binding sites per cell calculated at 100% inhibition of i_{MK}^P; turnover number means ions pumped per site per minute. The K^+ influxes were determined in 10 mM external K^+. The intracellular concentrations were determined after incubation of the cells at 37° for 1–2 h in K^+-free medium (freshly drawn cells have slightly higher $[K^+]_i$ and lower $[Na^+]_i$). See text for method and assumptions involved in calculation of turnover numbers. The individual adults, for which values are given, were involved in the parentage of the lambs. The mean values for adults were determined on sheep from the same flock, and were taken from ref. 6.

	Age	$[K^+]_i$	$[Na^+]_i$	i_{MK}^P	i_{MK}^L	*Total sites*	*Turnover number*
HK lambs	15	94	18	0.81	0.07	54	5060
	19	101	22	0.76	0.06	54	4750
HK adults		82	31	0.74	0.06	43	6000
		77	32	0.86	0.07	56	5160
(mean, 4 sheep)		76	27	0.88	0.06	48	6000
LK lambs	11	73	53	0.54	0.29	33	6000
	14	77	58	0.62	0.30	36	6200
	19	73	48	0.34	0.26	29	4430
LK adults		16	92	0.14	0.28	18	4250
		13	88	0.12	0.23	12	6000
(mean, 7 sheep)		14	94	0.11	0.23	14	4800

Table I shows the cation composition, pump and leak fluxes of K^+, and tota pump sites per cell for two HK and three LK lambs. Also shown are values for individual adult sheep involved in the parentage of the lambs, and mean values for a number of adult sheep from the same flock reported previously[6].

The turnover numbers (ions pumped per min per site) were calculated from pump fluxes, pump sites, and mean cell volume (30 μm^3), after a correction of total binding sites for six nonspecific sites (ouabain binding sites not involved in transport). This is based on the fact that cells exposed to Cs^+ during the incubation with [^{3}H]-ouabain require fewer ouabain molecules per cell for 100% inhibition of the pump[6], indicating selective inhibition of nonspecific binding by Cs^+. The number of non-specific sites per cell was about 6 for both HK and LK cells. However, in both HK and LK sheep there was considerable variation in the Cs^+ effect among individuals. In addition there is no information on the Cs^+-determined nonspecific sites on cells from lambs. Thus the turnover numbers for the lamb cells must be regarded as approximate.

The pump and leak fluxes of K^+ and the number of pump sites for cells from HK lambs are indistinguishable from those for cells from HK adults, although $[K^+]_i$ is slightly higher and $[Na^+]_i$ slightly lower in lambs. However, red cells of LK lambs are definitely intermediate between HK cells and adult LK cells with regard to pump flux, pump sites, and cation concentrations. Thus, the reduction in $[K^+]_i$ and pump flux during maturation of genetically LK lambs can be attributed at least in part to

a reduced number of pump sites per cell. This reduction in number of pump sites per cell could reflect either a replacement of cells in the circulation[16] or a conversion of the sites on cells remaining in circulation from pumps to leaks[6,7]. It is likely that both mechanisms are operative.

The data are generally consistent with the hypothesis that HK and LK cells differ in the fractions of total sites which are pumps and leaks. However, the relatively high leak fluxes in LK lamb cells are inconsistent. But if leak fluxes are partially mediated[6] these high leak fluxes might be due to an activating effect of the high $[K^+]_i$ on the leak influx of K^+.

The turnover numbers appeared to be about the same in all the sheep. This cannot be taken as evidence of identical kinetic properties for the transport sites from all the sheep since the different intracellular concentrations of K^+ and Na^+ may exert varying inhibitory and activating effects, respectively[17]. Nevertheless, the similarity in turnover numbers per site for red cells for all the sheep under more or less physiological conditions indicates that the reduction in number of pump sites may be the primary cause of the observed changes in cation composition and in pump fluxes in the LK lambs.

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