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Characteristics of Na^+-ATPase of low-K^+ sheep red cell membranes stimulated by blood group L antiserum

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SUMMARY

1. The effect of isoimmune (anti-L) serum on the Na^+-activated ATPase activity of low-K^+ (LK) type red cell membranes was studied at low ATP concentration.

2. The L-antigen–antibody reaction results in a marked increase in the velocity of Na^+-dependent ATP hydrolysis.

3. The response of anti-L-stimulated Na^+-ATPase in homozygous LL (low-K^+) membranes to K^+ resembles that of low-K^+ rather than high-K^+ (HK) membranes.

Recent studies of the comparative properties of high-K^+ (HK) and low-K^+ (LK) sheep red cells have revealed both quantitative and qualitative (kinetic) differences in the Na^+-stimulated ATPase activity of the membrane prepared from these two types of cells[1]. The activity of low-K^+ membranes was an order of magnitude lower than that of high-K^+ membranes and differed in its response to added K^+. In particular, low concentrations of K^+ inhibited but never stimulated the ATPase activity in low-K^+ membranes while with high-K^+ membranes, K^+ stimulates at lower and inhibits only at higher concentrations. This behaviour is similar to the reported differences in the response of the Na^+-K^+ pump of low-K^+ and high-K^+ cells to intracellular concentrations of Na^+ and K^+ (refs. 2, 3).

Treatment of low-K^+ red cells with specific isoimmune (anti-L)[4] sera has been shown to increase the intracellular K^+ level[5], the activity of the Na^+-K^+ pump[5,6], the (Na^+, K^+)-ATPase activity[6] and the number of [^{3}H]ouabain binding sites[5,6]. These observations prompted the present investigation which was designed to determine whether the anti-L-stimulated increment in alkali cation-sensitive ATPase responds to K^+ in a manner similar to that of low-K^+ membranes or that of high-K^+ membranes.

Isoimmune anti-L and non-immune sera were prepared as described previously[5]. The sera were then dialyzed and lyophilized. Sheep blood was obtained from homozygous low-K^+ (LL) or high-K^+ (MM) sheep and membranes were prepared from the washed red cells as described previously[1].

Treatment of membranes with isoimmune and non-immune sera was carried out as follows: 1 vol. membranes equivalent to 1 vol. packed red cells was mixed with 0.38 vol. serum (38 mg protein per ml dissolved in 0.53 M Tris-HCl, pH 7.4) so that the final Tris-HCl concentration was 0.15 M, and then allowed to incubate at 4° overnight. The membranes were then diluted to 40 vol. with 0.15 M Tris-HCl, pH 7.4, centrifuged at 35 000 × *g* for 15 min and the supernatant was discarded. The membrane pellet was suspended in 40 vol. 5 μM Tris-EDTA, pH 7.4, and centrifuged as before. The membranes thus obtained were diluted with 2 mM Tris-HCl, pH 7.4, containing 4 μM Tris-EDTA, pH 7.4, to the original packed cell volume (low-K^+ membranes) or to twice the original packed cell volume (high-K^+ membranes).

ATPase activity was measured using [γ-^{32}P]ATP as described previously[1,7]. The assay medium contained 0.2 μM ATP, in a final volume of 0.25 ml containing 0.1 ml membrane suspension. 50 mM NaCl and varying amounts of KCl were added as indicated. Incubation was for 2 min at 37°. Mg^{2+}-ATPase activity refers to the rate of ATP hydrolysis in the absence of NaCl, Na^+-ATPase, to the increment in rate effected by 50 mM NaCl.

TABLE I

EFFECT OF ANTI-L ON ATPase ACTIVITY OF LOW-K^+ AND HIGH-K^+ MEMBRANES

Membranes were treated with sera and assayed as described in the text. In Expt. 1, 0.154 M Tris-HCl, pH 7.4, replaced serum.

Expt. No.	*Cells*	*Serum*	*ATPase activity (pmoles $^{32}P_i$ released per mg per min)*	
			Mg^{2+}-ATPase	*Na^+-ATPase*
1	Low-K^+ (284)	Anti-L	15.9	35.7
		Non-immune	14.1	7.8
		(None)	14.6	9.5
	High-K^+ (76W)	Anti-L	23.5	58.5
		Non-immune	15.5	46.6
		(None)	17.6	64.9
3	Low-K^+ (284)	Anti-L	13.5	37.0
		Non-immune	11.2	7.6
	High-K^+ (76W)	Anti-L	16.0	53.7
		Non-immune	13.8	54.6
5	Low-K^+ (241)	Anti-L	15.7	13.9
		Non-immune	11.7	4.0
	High-K^+ (72Y)	Anti-L	10.1	33.6
		Non-immune	10.1	52.9

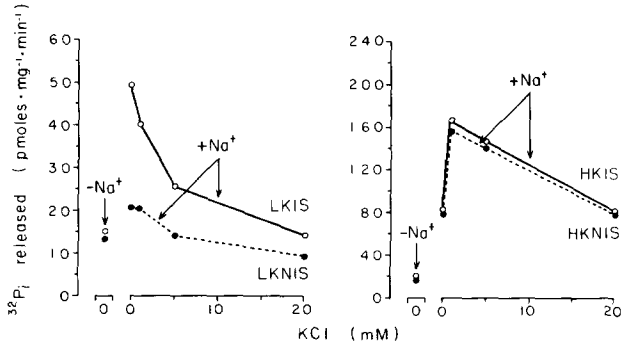

Fig. 1. Effect of anti-L serum on high-K^+ and low-K^+ red cell membrane Na^+-ATPase activity. Homozygous LL (low-K^+) and high-K^+ membranes were treated with either non-immune serum, designated LKNIS and HKNIS, respectively (●---●), or isoimmune anti-L serum, designated LKIS and HKIS, respectively (○——○). The ATPase activity was then determined without NaCl or with 50 mM NaCl *plus* 0, 1, 5 and 20 mM KCl, as indicated. The data shown are the average of two experiments with two separate preparations from one LL (low-K^+) sheep and one high-K^+ sheep.

Table I and Fig. 1 show the Na^+-ATPase activity of low-K^+ and high-K^+ membranes treated with non-immune and immune sera and then assayed in the absence and presence of varying amounts of K^+. In the absence of K^+ (Table I), the Na^+-ATPase activity of low-K^+ membranes was stimulated 3- to 5-fold by anti-L serum, to a value 25–75% that observed with either high-K^+ membranes treated with nonimmune serum or high-K^+ membranes treated with isoimmune serum. Upon addition of increasing amounts of K^+ (1, 5 and 20 mM) (Fig. 1), the Na^+-ATPase activity of both low-K^+ membranes treated with non-immune serum and low-K^+ membranes treated with isoimmune serum was inhibited at all concentrations of K^+ tested. In contrast, Na^+-ATPase of either high-K^+ membranes treated with non-immune serum or high-K^+ membranes treated with isoimmune serum was stimulated by small amounts of K^+. Maximal activity, observed with 1–5 mM K^+, was decreased at 20 mM K^+ to a value equal to that observed in the absence of K^+.

In order to facilitate comparison of the response to K^+ of the ATPase activity in the two different types of membranes the activity at each K^+ level was expressed as a percentage of the activity observed in the absence of added K^+. Fig. 2 shows the percent activity so defined plotted as a function of K^+ concentration for high-K^+ and low-K^+ membranes and for the increment in ATPase activity produced in low-K^+ membranes by anti-L. The results of five separate experiments, each with a different preparation, are shown in Fig. 2. The data indicate that the response of the antibody-stimulated Na^+-ATPase to K^+ is closely similar to that observed in low-K^+ rather than high-K^+ membranes.

The membrane asymmetry for optimal activation of ATP hydrolysis by Na^+ at the inside and K^+ at the outside is lost when broken membrane preparations are assayed. However, assays of ATPase activity at very low concentrations of ATP do reflect to some extent the separate effects of Na^+ and K^+, *i.e.* Na^+ alone stimulates ATP hydrolysis in either human[8] or sheep red cell membranes[1,9]. Furthermore in these systems, Na^+-dependent ATPase activity is stimulated by low but inhibited by high concentrations of K^+.

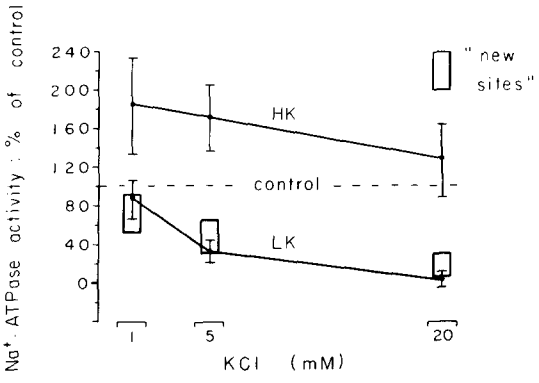

Fig. 2. Comparison of K^+ response curves of high-K^+, low-K^+ and L-antigen–antibody-stimulated 'sites'. Na^+-ATPase activity was determined as described in Fig. 1. For each concentration of KCl tested, the ATPase activity was measured without and with 50 mM NaCl. Na^+-dependent ATPase thus measured at 1, 5 and 20 mM KCl is expressed as a percentage of Na^+-ATPase measured without added KCl. The results (mean ± S.D.) for six high-K^+ preparations treated with either non-immune or immune serum and five low-K^+ preparations treated with non-immune serum are shown by the curves labelled HK and LK, respectively. The increment in Na^+-ATPase produced in low-K^+ membranes by anti-L (see Fig. 1), *i.e.* 'new sites', determined with 1, 5 and 20 mM KCl are expressed also as a percentage of the anti-L-stimulated increment observed in the absence of added KCl and are represented by the open rectangles.

By contrast, even low concentrations of K^+ inhibit the Na^+-dependent ATPase activity in low-K^+ membranes. These properties of the membrane ATPase system correlate well with the greater sensitivity of the low-K^+ sheep red cell membrane active transport system to inhibition by internal K^+ (ref. 3).

Stimulation of low-K^+ Na^+-ATPase by anti-L was similar in magnitude (approx. 4-fold) to the stimulation of low-K^+ pump activity described previously[5,6]. In the former study[3], the kinetic characteristics of the new pump sites resembled homozygous LL (low-K^+) cells with respect to the kinetics of activation (K_m) by extracellular K^+, but resembled MM (high-K^+) cells with respect to response to varying concentrations of intracellular Na^+ and K^+.

The results of the present study show that the L-antigen–antibody reaction results in an increase in the velocity of Na^+-dependent ATP hydrolysis at low ATP concentrations but does not alter the response of this activity in LL (low-K^+) membranes to K^+. This result is consistent with the conclusion that the reaction between anti-L and the L-antigen increases the number of active transport sites in low-K^+ sheep red cell membranes. These 'new sites' have kinetic characteristics similar to the sites normally present in low-K^+ membranes. This conclusion is clearly different from that noted above which was drawn from studies of the active transport system in intact low-K^+ sheep red cells[2,3,5]. Definition of the origin of this difference will require further investigation.

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