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REVERSAL OF THE EFFECTS OF A LOW EXTRACELLULAR POTASSIUM CONCENTRATION ON THE NUMBER AND ACTIVITY OF Na^+/K^+ PUMPS IN AN EPSTEIN–BARR VIRUS-TRANSFORMED HUMAN LYMPHOCYTE CELL LINE

MOHAMMED AMEEN,* JANET G. BLOOMFIELD and JEFFREY K. ARONSON
MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

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Abstract—A reduction in the extracellular concentration of potassium to 0.5 mM (low K) in Epstein–Barr (EB) virus-transformed lymphocytes caused changes in the number and activity of Na^+/K^+ pumps in the cell membrane, with increases in the B_{max} and apparent K_d of ouabain binding, and concomitant increases in the V_{max} and apparent K_m of potassium (rubidium) influx. However, recovery from the effects of low K occurred more quickly than the original up-regulation. Furthermore, there were differences in the time-courses of the separate rates of recovery of the B_{max} and K_d of ouabain binding after the cells were returned to normal K, the rate of recovery of the K_d being quicker than that of the B_{max}, which was biphasic, with slow and fast rates of recovery. Inhibition of protein synthesis by emetine caused an increase in the rate of recovery of the B_{max} of ouabain binding, but no effect on the K_d, suggesting that the slow phase of recovery of the B_{max} is attributable to the synthesis and insertion of new protein, while the rapid phase of recovery is independent of protein synthesis and may represent internalization. The results suggested that during up-regulation of pump number in response to low K about 40% of the newly inserted Na^+/K^+ pumps are normal and the rest are abnormal. The half-time of removal of the abnormal pumps from the cell membrane during recovery from low K stress was 2.8 hr and the half-time of internalization of the normal pumps was 4.3 hr.

The Na^+/K^+-ATPase (Na^+/K^+ pump, EC 3.6.1.37) is a bi-subunit, tetrameric, integral membrane protein that maintains the electrochemical gradients of sodium and potassium ions across the plasma membranes of all animal cells. Its activity is essential in the maintenance of numerous cellular processes [1] and is subject to regulation in response to numerous stimuli.

The Na^+/K^+ pump is also the pharmacological receptor for the cardiac glycosides [2], and the cardiac glycoside-binding site, the phosphorylation site and the Na^+ and K^+ binding sites have all been shown to be on the α subunit of the protein. The role of the β subunit seems to be to anchor the pump to the plasma membrane [3]. Recent work has shown that it regulates the abundance of the Na^+/K^+ pump [4] and that it is involved in the transportation of the α subunit from the endoplasmic reticulum to the plasma membrane.

The regulation of the expression of the Na^+/K^+ pump has been studied in a variety of *in vitro* and *in vivo* experimental systems. Among the most extensively studied of these is the up-regulation in the number of Na^+/K^+ pumps which occurs in HeLa cells [5, 6], primary chick heart cells [7], ARL 15 cells [8], and lymphocytes [9,10] in response to a reduction in the extracellular concentration of potassium (low K). Other stimuli which cause up-regulation in HeLa cells and lymphocytes include lithium [9, 12], ethacrynic acid [9, 12], cardiac glycosides and aglycones (ouabain [12] and acetylstrophanthidin [13]), monensin [10, 14], thyroid hormones [15], glucocorticoids [16], and mineralocorticoids [17].

There is some evidence that when up-regulation of Na^+/K^+-ATPase occurs in response to perturbations of concentrations of sodium and potassium it occurs via different mechanisms from those operating in hormone-mediated up-regulation. In MDCK cells, HeLa cells and lymphocytes a rise in intracellular sodium concentration has been proposed as the trigger for the up-regulation in Na^+/K^+ pump number which occurs when the cells are exposed to a low external concentration of potassium [7, 10, 12]. A similar mechanism has been proposed for the up-regulatory effects of cardiac glycosides, ethacrynic acid, and monensin [10]. On the other hand, direct induction of the transcription of the Na^+/K^+-ATPase proteins via hormone–receptor complexes has been postulated as the mechanism for Na^+/K^+ pump up-regulation by hormones [16].

In general the net increases in the accumulation of Na^+/K^+ pump sites due to perturbations in ion concentrations or altered concentrations of hormones seem to result through secondary increases in the α_1, α_2, and/or β subunit mRNAs. However, the rate of onset and the relative degrees of up-regulation of the α and β mRNAs, and therefore of the amounts of the corresponding functional pump proteins synthesized, vary both between cell types and with the type of stimulus. In addition, it is possible to distinguish cells depending on whether they up-

* Corresponding author. Tel. (0865) 241091; FAX (0865) 791712.

regulate slowly or rapidly. Using Epstein–Barr (EB*) virus-transformed human lymphocytes we have previously shown a 35% increase in membrane Na^+/K^+ pump number in response to low K [10], but this effect did not occur until the cells had been exposed to low K for 72 hr. In contrast, in HeLa cells [5] and chick myoblasts [7] up-regulation occurs rapidly; in MDCK cells an increase in the number of Na^+/K^+ pump sites occurred within 24 hr of the start of low K stress [18] and at the same time there was a 3-fold induction in the α_1 and β subunit mRNAs. The maximum rate of induction occurred within 1 hr.

The processes and the mechanisms of the recovery of cells from the up-regulated state after removal of the up-regulating stimuli have not been studied in detail. In HeLa cells a return to normal K resulted in a rapid reduction in the number of Na^+/K^+ pump sites in the plasma membrane [6]. This rapid reduction in cell membrane Na^+/K^+ pump number has also been shown for veratridine-induced up-regulation in chick muscle cells [14]. Both of these cell types up-regulate rapidly and the kinetics of recovery are even more rapid.

In contrast, EB virus-transformed lymphocytes up-regulate slowly [10], and we have therefore studied the effects of replacing a low extracellular K concentration with normal extracellular K on the number and activity of Na^+/K^+ pumps in EB virus-transformed lymphocytes. We have also outlined the time-course of recovery of the cells from up-regulation after the removal of the up-regulating stimulus (low K) and the effects of inhibition of protein synthesis on that time-course. We have previously presented some of these results in abstract elsewhere [19].

MATERIALS AND METHODS

Media and antibiotics for cell culture were obtained from Flow Laboratories (Irvine, U.K.) and fetal calf serum from Gibco (Uxbridge, U.K.). Amersham International (Amersham, U.K.) supplied ^{86}RbCl and New England Nuclear (Boston, MA, U.S.A.) supplied [G-^{3}H]ouabain. All other chemicals were obtained from the Sigma Chemical Co. (Poole, U.K.).

Cell culture conditions. The lymphoblast cell line B1 was obtained by transforming healthy adult peripheral lymphocytes with EB virus using standard techniques [20]. B1 cells were maintained in stationary suspension culture at 37° and 100% humidity in an atmosphere of air/CO_2 (95%/5%) in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 10^5 I.U./L penicillin, and 100 mg/L streptomycin. The cells were sub-cultured twice weekly and cell densities were kept at between 3×10^5 and 1.2×10^6 cells/mL. Under these conditions the doubling time was around 45 hr.

Up-regulation due to low K. In experiments in which the extracellular potassium concentration was reduced the cells were cultured in 10% (v/v) dialysed fetal calf serum and potassium-free RPMI 1640 medium with added KCl to a final concentration of potassium of 0.5 mM. Two sets of transformed lymphocytes were grown in either 5.0 mM potassium (normal K) or 0.5 mM potassium (low K) for 72 hr. Specific [^{3}H]ouabain binding and ^{86}rubidium (^{86}Rb) influx were measured after 72 hr as described below.

Recovery from up-regulation after return to normal K. After 72 hr cells which had been incubated with potassium concentrations of 5.0 and 0.5 mM were washed and resuspended in RPMI 1640 with 10% (v/v) dialysed fetal calf serum and 5.0 mM K for further periods of 2–48 hr (recovery). Recovery was measured at eight time-points, 2, 4, 8, 16, 20, 24, 44 and 48 hr. Specific [^{3}H]ouabain binding was measured on each occasion as described below. ^{86}Rb influx was measured at 24 hr after recovery.

The effects of inhibition of protein synthesis on the recovery from up-regulation after return to normal K. The first part of this experiment was done as outlined above. After 72 hr in either normal K or low K, the cells were washed twice in normal K or low K medium, respectively, and then resuspended for 2, 4, 8, 24, or 48 hr in either normal K RPMI with dialysed fetal calf serum or low K RPMI with dialysed fetal calf serum containing $3.5 \times 10-8$ M emetine. Specific [^{3}H]ouabain binding was measured on each occasion as described below. Cell viability, measured by Trypan blue exclusion, was greater than 95% in cells which had been incubated with or without emetine.

Measurement of [^{3}H]*ouabain binding to intact transformed lymphocytes.* At the end of each experimental period the cells were harvested by centrifugation at 700 *g* for 15 min at room temperature. The cells were washed once and resuspended in 5.0 mL of potassium-free Ringer solution just before the assay. [^{3}H]Ouabain binding was measured at seven concentrations of [^{3}H]ouabain from 5 to 40 nM in a total incubation volume of 500 μL and allowed to proceed at 37° for 90 min, by which time binding had reached equilibrium at all the concentrations of ouabain used. The cell count in each assay tube was kept at between 5×10^5 and 1×10^6 cells. Bound ouabain was separated from unbound ouabain by centrifugation at 2000 *g* and 4° for 5 min. The cells were washed by resuspending them in 750 μL of ice-cold Ringer solution and they were then pelleted by centrifugation as above. The pellet was digested in 500 μL of 1.0 M NaOH and 400 μL of the resulting solution was used for radioactivity counting. Where necessary 50 μL of this solution was used for protein estimation. Non-specific binding of [^{3}H]ouabain was estimated with 10^{-4} M non-radioactive ouabain and was always less than 1% of the total amount of ouabain bound. The results have been expressed as fmol of ouabain bound per 10^6 cells (B_{max}) and nM (K_d). The number of Na^+/K^+ pump sites per cell was calculated on the assumption that one molecule of ouabain binds to each pump site.

Measurement of rubidium influx into intact transformed lymphocytes. Cells were prepared as described above. Rubidium influx was measured at six concentrations of non-radioactive rubidium (between 0.6 and 6.0 mM), supplemented with ^{86}Rb (1.0 μM). Non-glycoside-sensitive influx of rubidium was estimated by using 0.1 mM ouabain to block Na^+/K^+ pump-dependent influx, which was around

* Abbreviation: EB, Epstein–Barr.

Table 1. The K_d and B_{max} of [^{3}H]ouabain binding in cells which had been incubated in normal K (5 mM) or low K (0.5 mM) for 72 hr and after further incubation in both cases in 5 mM K (recovery) for 24 hr

Experimental condition	K_d (nM)	B_{max} (fmol/10^6)	Na^+/K^+ pump sites per cell
Effects of up-regulation			
Normal K 72 hr	20.1 (1.5)	1769 (204)	1.06 (0.30) × 10^6
Low K 72 hr	23.5 (1.7)*	2563 (286)*	1.54 (0.42) × 10^{6*}
Effects of recovery			
Normal recovered	19.8 (1.7)	1700 (139)	1.02 (0.20) × 10^6
Low K recovered	20.7 (1.4)	1970 (172)†	1.16 (0.25) × 10^6

* $P < 0.05$, † $P = 0.06$. N = 11 in each case.

70% of total influx. In a typical assay between 5×10^5 and 1×10^6 cells per tube were incubated with rubidium for 5 min at 37° in a total volume of 500 μl of potassium-free Ringer solution. The uptake of rubidium into transformed lymphocytes under these conditions was linear for 15 min. Uptake was stopped by rapidly cooling the cells to 0° in ice. The cells were centrifuged at 4° and 2000 *g* for 15 min to remove extracellular rubidium. The cell pellet was washed and resuspended in potassium-free Ringer, and intracellular radioactivity was determined as outlined above. The K_- and V_{max} of rubidium uptake were calculated using semi-reciprocal square-root plots, assuming two external binding sites for rubidium per Na^+/K^+ pump site [21]. The turnover rate of the pump (the number of ions transported per pump site per minute) was calculated by dividing the V_{max} of rubidium influx by the B_{max} of ouabain binding.

Statistical analyses. The data are presented as means (SE). Statistical comparisons have been made by paired two-tailed Student's *t*-tests.

RESULTS

The effects of a reduction in the extracellular concentration of potassium on the number and activity of Na^+/K^+ pumps of transformed human lymphocytes

Ouabain binding. Table 1 shows the effect on ouabain binding of incubating the transformed lymphocytes in an extracellular potassium concentration of 0.5 mM for 72 hr. There was a 45% increase in the B_{max} of ouabain binding and the number of pump sites per cell increased significantly from 1.06×10^6 to 1.54×10^6 ($P < 0.05$). These results confirm our previous findings [10].

In addition, the apparent dissociation constant (K_d) of cells which had been exposed to low K increased significantly from 20.1 (3.5) to 23.5 (4.2) nM ($P < 0.05$). This has not been reported before.

Rubidium influx. Table 2 shows the effect of incubating the transformed lymphocytes in an extracellular potassium concentration of 0.5 mM for 72 hr on rubidium influx. Both the maximum rubidium uptake capacity (V_{max}) and the apparent affinity (K_m) of the pump for rubidium increased significantly, the V_{max} by 50% [from 3.96 (1.67) to 5.98 (1.94) fmol/cell/min], the K_m by 30% [from 0.62 (0.20) to 0.81 (0.25) mM] (both $P < 0.05$). These results also confirm our previous findings [10].

There was no change in the turnover rate of the pump, i.e. the number of rubidium ions transported per Na^+/K^+ pump site per minute, after incubation in low K for 72 hr.

The effects of returning the transformed lymphocytes to normal extracellular potassium

The effects of recovery for 24 hr. When the transformed lymphocytes which had been incubated in low K for 72 hr were returned to normal K for 24 hr there was a complete reversal of the changes in the K_d of ouabain binding (Table 1) and in the K_m and V_{max} of rubidium influx (Table 2). The B_{max} of ouabain binding returned towards normal, but was still 16% above pre-recovery values at 24 hr ($P = 0.06$) (Table 1). Recovery from low K stress did not affect the turnover rate of the pump (Table 2).

The time-course of recovery of ouabain binding sites. The time-course of recovery of ouabain binding sites over 48 hr after the return to normal K is shown in linear co-ordinates in Fig. 1 and in a semi-log plot in the inset to the figure. There was an initial rapid phase of recovery followed by a much slower phase of recovery. Full recovery did not occur until 44–48 hr.

In contrast, the K_d of ouabain binding had returned to normal by 4 hr of returning the cells to normal K (Fig. 2).

The effect of inhibition of protein synthesis on the time-course of recovery of ouabain binding sites. We used 3.5×10^{-8} M emetine dihydrochloride to inhibit protein synthesis. The results showed that the B_{max} recovered by 24 hr in the presence of emetine (Fig. 3), i.e. more quickly than in the absence of emetine (Fig. 1). On the other hand, emetine had no effect on the time-course of recovery of the K_d (data not shown).

DISCUSSION

Changes in Na^+/K^+ pump numbers and activity after exposure to low K

When EB virus-transformed lymphocytes are

Table 2. The K_m and V_{max} of rubidium influx in cells which had been incubated in normal K (5 mM) or low K (0.5 mM) for 72 hr and after further incubation in both cases in 5 mM (recovery) for 24 hr

Experimental condition	K_m (mM)	V_{max} (f mol/cell/min)	Turnover rate of pump (ions/site/min)
Effects of up-regulation			
Normal K 72 hr	0.62 (0.06)	3.96 (0.37)	2239 (125)
Low K 72 hr	0.81 (0.08)*	5.98 (0.66)*	2333 (139)
Effects of recovery			
Normal K recovered	0.69 (0.09)	4.66 (0.38)	2741 (317)
Low K recovered	0.71 (0.08)	4.44 (0.53)	2254 (333)

* $P < 0.05$; N = 9 in each case.

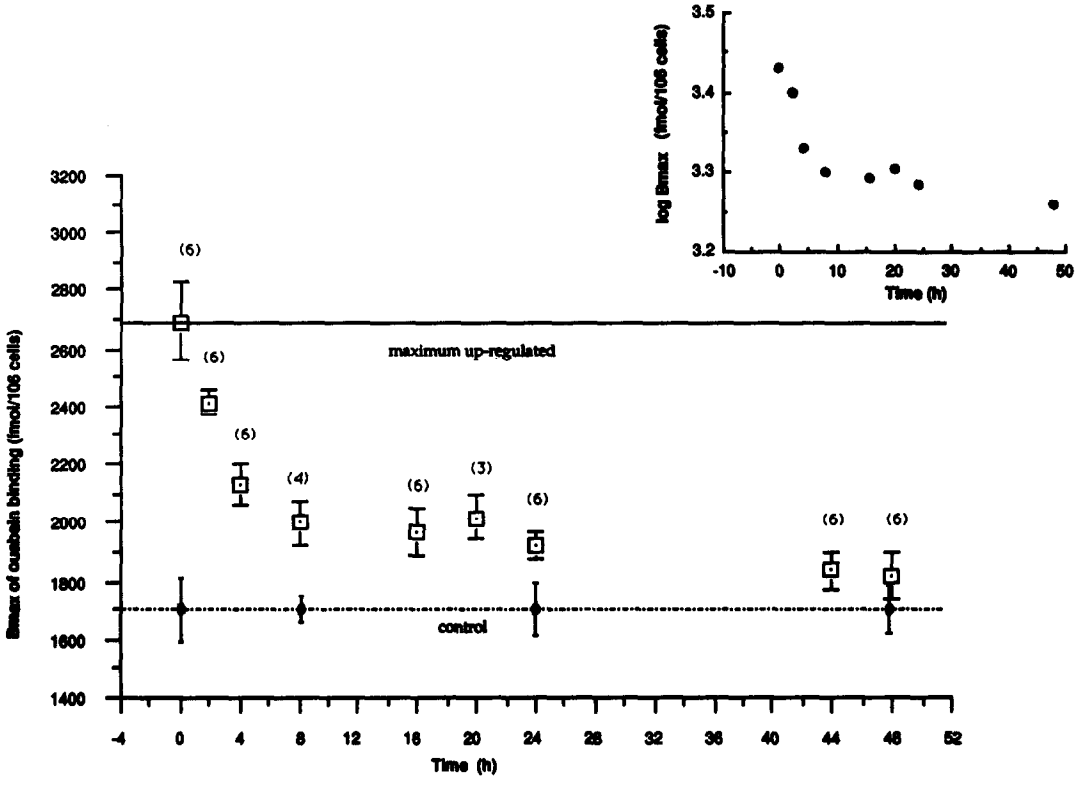

Fig. 1. The rate of recovery of the B_{max} of ouabain binding to EB virus-transformed lymphocytes after the removal of low K stress. The numbers of experiments at each time point are shown in parentheses. The fitted line was developed on a Macintosh computer using Regression (Blackwells Scientific Software, Oxford). The inset shows the mean data in semi-logarithmic plot.

exposed to a low external concentration of potassium (0.5 mM) for 72 hr the number of Na^+/K^+ pumps in the cell membrane increases, with a parallel proportionate increase in the maximum rate of potassium influx (measured here using rubidium). These results confirm our previous findings [10]. We have also confirmed that these changes are accompanied by a decrease in the apparent affinity of the external binding site of the pump for potassium (measured here as the K_m of rubidium influx).

We have now extended these findings by showing that there is a concomitant reduction in the apparent affinity of the Na^+/K^+ pumps for ouabain (i.e. an increase in K_d).

Since the ouabain binding site and the potassium binding site are both on the α subumit of the Na^+/K^+ pump, these results are compatible with a change in the nature of the α subunit. This could be due to a change in the isoform of the subunit, since it has been reported that the α_1 subunit of rat Na^+/K^+-ATPase has a lower apparent affinity for both ouabain and sodium than the $\alpha 2$ subunit [22].

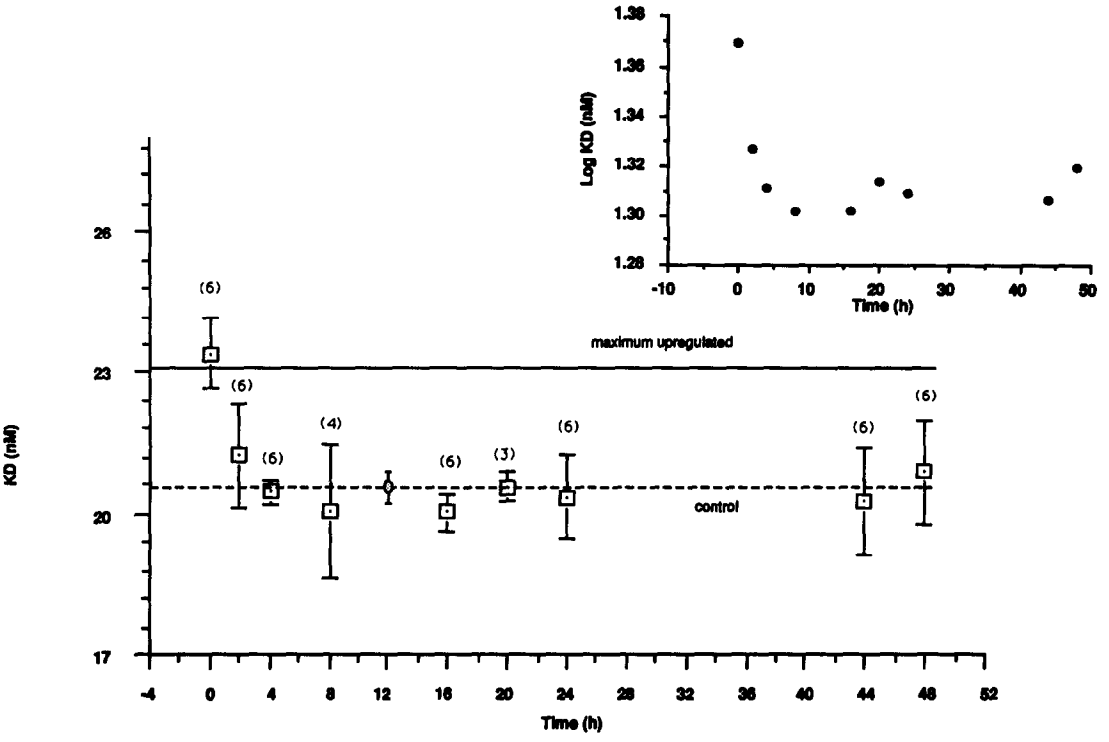

Fig. 2. The rate of recovery of the K_d of ouabain binding to EB virus-transformed lymphocytes after the removal of low K stress. The numbers of experiments at each time point are shown in parentheses. The fitted line was developed on a Macintosh computer using Regression (Blackwells).

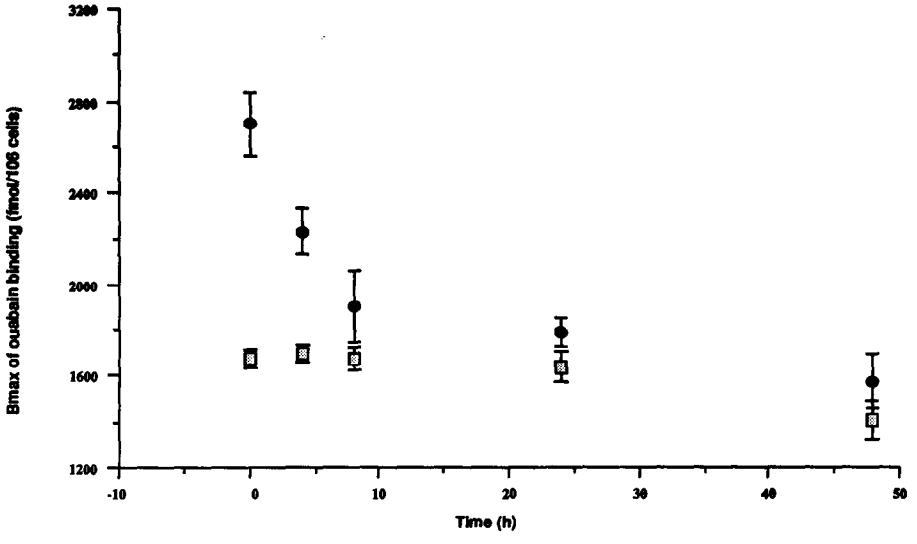

Fig. 3. The effect of inhibition of potein synthesis with 3.5×10^{-8} M emetine on the recovery of the B_{max} of ouabain binding to EB virus-transformed lymphocytes after removal from low K stress (●) and in transformed lymphocytes incubated in normal K (▨) (N = 5).

However, it is known that an up-regulation in Na^+/K^+ pumps can occur without a change in the isoform of the α subunit, since in ARL 15 cells only the α_1 subunit is detected both in normal cells and in cells which have up-regulated in response to low K [23]. Alternatively, therefore, there might be a small structural change in the α subunit in transformed lymphocytes, resulting in a change in ouabain affinity

without a change in isoform, for example due to post-translational processing or a conformational change in the α subunit [24]. Using one-dimensional denaturing polyacrylamide gel electrophoresis we have not been able to show any gross changes in the size of the α subunit between low K-treated and normal transformed lymphocytes (data not shown), but in this context it is of interest to note that the α subunit can be changed from an ouabain-sensitive form to an ouabain-resistant form by changing the surface charge of the first externally facing segment of the α subunit, by replacing glutamine and asparagine with arginine and aspartate using site-directed mutagenesis [25].

Since the β subunit is necessary for anchorage of the α subunit in the cell membrane, a further alternative explanation is that a change in the nature of the β subunit might cause secondary changes in the binding of ouabain and potassium to the α subunit. However, transfectional experiments using chick β subunit expression in mouse cells have shown that structural changes in the β subunit do not directly change ouabain binding affinity [24]. The β subunit has been shown to be encoded by multiple sizes of mRNA that seem to arise out of the use of different polyadenylation sites [26], but are all transcribed from the same gene. However, mRNA from this gene has not been detected in tissues such as liver [27], and this suggests that there may be other isozymes of the β subunit which could influence the binding of ouabain to the α subunit.

Changes in the apparent affinity for ouabain binding need not be restricted to isozyme and subunit effects. Several intracellular and cytoskeletal factors have also been proposed. For example, in MOPC MF_2S cells ouabain affinity changed from 100 to 1 μM when EDTA was added to the membrane preparation [28, 29], suggesting that calcium has an effect on protein–protein interactions of the α subunit with other supramolecular structures at the cytoplasmic face in the murine plasmacytoma cell line.

We are currently carrying out experiments to investigate these possibilities.

The time-course of reversal of the effects of low K

The K_d of ouabain binding and the V_{max} and K_m of rubidium influx had all returned to normal by 24 hr of returning the cells to normal K (Tables 1 and 2). However, the B_{max} of ouabain binding had not completely returned to normal by that time. We therefore studied the time-course of reversal of the effects of low K on the B_{max} and K_d of ouabain binding over 48 hr (Figs 1 and 2).

There was an initial rapid return of B_{max} towards normal during the first 8 hr, followed by a slower phase of recovery (Fig. 1). Normal B_{max} was restored only after 44–48 hr. In contrast, the recovery of the K_d to control values had only a single phase (Fig. 2). We fitted the K_d data to the following equation:

$$K(t) = K(0)e^{-kt} + K(c) \qquad (1)$$

where $K(t)$ is the K_d at any time t during recovery, $K(0)$ is the K_d at the beginning of the experiment (i.e. after exposure to low K), and $K(c)$ is the K_d at the final plateau (i.e. under control conditions). The rate constant of recovery of K_d (k in Eqn 1) was 0.746 (0.715) hr^{-1}, corresponding to a half-time of 0.93 (0.89) hr.

The return to normal of the K_d of ouabain binding by 4 hr suggests that all abnormal pumps had been removed from the cell membrane by that time, and this is consistent with the apparent half-time of 0.93 hr. None the less, the number of Na^+/K^+ pumps at 4 hr was still increased by 40% above normal. This suggests that when new pumps are inserted in the cell membrane during exposure to low K, about 40% are normal pumps, the remainder being pumps of abnormal structure, as discussed above.

The fact that the half-time of recovery of pump numbers was considerably slower than the half-time of recovery of the K_d suggests that the rate of removal of abnormal pumps differs from that of normal pumps and that abnormal pumps are removed more quickly. Were this so we would have expected to have seen a biphasic decline in the numbers of pumps during recovery. We therefore fitted the data shown in Fig. 1 to the following relationship:

$$B(t) = B(0)_1 e^{-kt_1} + B(0)_2 e^{-kt_2} + B(c) \qquad (2)$$

where $B(t)$ is the amount bound at any time t during recovery, $B(0)_1 + B(0)_2 = B(0)$, the total amount bound at the beginning of the experiment (i.e. after exposure to low K), and $B(c)$ is the amount bound at the final plateau (i.e. under control conditions).

The results yielded rate constants of 0.247 (0.093) and 0.00596 (0.0236) hr^{-1}. The first of these values corresponds to a half-time of 2.8 (1.1) hr However, the estimate of the second half-time has a very large standard error, and although it may be that the half-time of removal of normal pumps in these cells is very long we cannot make a quantitative estimate of that rate with any degree of certainty.

The half-time of 2.8 hr is similar to the value derived by Pollack *et al.* [6] in HeLa cells and by Lobaugh and Lieberman [quoted in 14] in cardiac cells, both of which are cells which up-regulate quickly in response to low K, in contrast to transformed lymphocytes, which up-regulate slowly (see introduction). This suggests that the overall rate of removal of pumps from the cell membrane is not related to the rate of up-regulation during low K stress. However, those workers did not identify a slower phase of recovery in their cells, and it may be that the rate of turnover of normal sites in EB virus-transformed lymphocytes is very slow and is related to the slow rate of up-regulation in these cells.

The effect of inhibiting protein synthesis on the time-course of reversal of the effects of low K

The half-time of recovery of pump number is related to two separate phenomena, the rate of internalization of pumps from the cell membrane and the rate of synthesis and insertion of newly synthesized pumps. The time-course of ouabain binding to the membrane (Fig. l) must represent the net effect of these two opposing effects. When protein synthesis was inhibited the B_{max} of ouabain binding recovered to control values by 24 hr, in comparison to 44–48 hr in the absence of the inhibitor, and the rate of recovery was monophasic

with a half-time of 4.3 (1.4) hr. Thus, the slow phase of recovery in the absence of emetine may represent a delay caused by the synthesis and insertion of new protein, while the rapid phase of recovery probably involves a process that is independent of protein synthesis and may represent internalization. This is similar to HeLa cells [6] and chick muscle cells [14], in which internalization was more important in recovery from an up-regulated state, whereas modulation of the rate of synthesis was shown to play a minor role.

The data also suggest that emetine may inhibit the internalization of newly formed pumps after exposure to low K, since the initial half-time of removal of pumps was prolonged from 2.8 to 4.3 hr. This is supported by evidence in HeLa cells that the internalization of Na^+/K^+ pumps can be inhibited by cycloheximide [5], although cycloheximide did not inhibit endocytosis in another receptor-mediated system, that of low-density lipoprotein [30].

Conclusions

We have confirmed that in EB virus-transformed lymphocytes there is an increase in the number and maximum activity of the Na^+/K^+ pumps in response to low K and that this effect is accompanied by an increase in the apparent K_d of ouabain binding and of the apparent K_m of potassium influx. These results suggest that at least some of the increased number of pumps which is inserted into the membrane are of abnormal structure or function. The differences in the time-courses of the separate rates of recovery of the B_{max} and K_d of ouabain binding suggest that during up-regulation of pump numbers about 40% of the newly inserted Na^+/K^+ pumps are normal and the rest are abnormal. The effects of emetine on the recovery rate of the B_{max} of ouabain binding suggest that the recovery of pump numbers after up-regulation occurs as a balance between a fast rate of internalization and a delay due to synthesis and insertion of new pumps into the membrane. The half-time of removal of the abnormal pumps from the cell membrane during recovery from low K stress was biphasic, the fast phase having a half-time of about 2.8 hr and the slow phase having too long a half-time for us to determine accurately. The half-time of internalization was 4.3 hr.

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