Biochimica et Biophysica Acta, 1027 (1990) 218–224
Elsevier

BBAMEM 74974

The effects of a low extracellular concentration of potassium on the activity and numbers of Na^+/K^+ pumps in an EB-virus transformed human lymphocyte cell line

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(Received 13 February 1990)
(Revised manuscript received 22 May 1990)

Key words: Potassium concentration; ATPase, Na^+/K^+-; Ouabain; (Human lymphocyte)

The BM1A EB-virus transformed human lymphocyte cell line contains approximately 950000 Na^+/K^+-ATPase sites per cell. The turnover number of each site is approx. 2240 molecules of rubidium per min. When cells are exposed to a low extracellular concentration of potassium the intracellular concentration of sodium rises, and the cells respond in the short term by increasing the V_{max} of $^{86}Rb^+$ uptake. In the longer term the cells respond by increasing both the V_{max} of $^{86}Rb^+$ uptake and the B_{max} of [^{3}H]ouabain binding. The suggestion that increases in the intracellular concentration of sodium is responsible for these changes is supported by the finding that monensin, which increases intracellular sodium without affecting intracellular potassium, is capable of inducing both the short- and long-term changes associated with a low external concentration of potassium.

Introduction

The sodium-potassium activated adenosine triphosphatase (Na/K-ATPase) is important in the maintenance of transmembrane concentration gradients of sodium and potassium. The number of Na/K-ATPase sites in the cell membrane is subject to regulation, and may be altered by several stimuli, including ouabain [1], ethacrynic acid [2,3], lithium [3,4], thyroid hormone [5], and a low extracellular concentration of potassium [3,6,7,8]. An earlier study from this laboratory [3] demonstrated that when the extracellular potassium concentration, $[K]_0$, was reduced the B_{max} of ouabain binding to human lymphocytes increased by 35% during a 72 h treatment. Similar results have been reported for HeLa cells [6] and MDCK cells [7]. In the last case it was shown that an increase in the intracellular sodium concentration, $[Na]_i$, may be the trigger for increasing the number of Na/K-ATPase molecules in the cell membrane. In addition, potassium depletion has been shown to increase the numbers of ouabain binding sites on erythrocytes [8] and the activity of the Na/K-ATPase in guinea pig heart in vivo [9], although the latter may not be accompanied by an increase in ouabain binding sties [8]. However, in a number of other in vivo studies it has been demonstrated that potassium depletion causes a decrease in the B_{max} of ouabain binding to the skeletal muscle of guinea pigs [8] and rats [10–12].

The present work was initiated to study the effects of decreased $[K]_0$ on the binding of [^{3}H]ouabain and the uptake of ^{86}Rb by a human lymphoblast cell line, BM1A. A preliminary account of part of this work has already been published [13].

Materials and Methods

Media, antibiotics, and sera for cell culture were obtained from Flow Laboratories Ltd. Kits for the determination of the activity of 5′-nucleotidase (catalogue number 675-PB), together with rubidium chloride and the substrates for the determination of the activity of γ-glutamyl transferase were obtained from the Sigma Chemical Company. Amersham International plc supplied D-[U-^{14}C]sorbitol, tritiated water (catalogue number TRS.3), and ^{86}RbCl. New England Nuclear Research Products supplied [G-^{3}H]ouabain.

Cell culture conditions

The BM1A cell line was obtained by transforming with Epstein-Barr virus the peripheral lymphocytes from a healthy adult using standard techniques [14]. BM1A

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cells were maintained in stationary suspension culture, using RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 100 IU · ml^{-1} penicillin and 100 $\mu g \cdot ml^{-1}$ streptomycin. The cells were incubated at 37°C in a humidified air/CO_2 (95 : 5, v/v) atmosphere. The cells were subcultured twice weekly and cell densities were kept between $2 \cdot 10^5$ and $1.5 \cdot 10^6$ cells · ml^{-1}. Under these conditions the cells had a population doubling time of 35–45 h. In experiments in which $[K]_0$ was reduced the cells were cultured in potassium-free RPMI-1640 supplemented with 10% (v/v) dialysed fetal calf serum, appropriate amounts of potassium chloride, and antibiotics as described above.

Measurement of [^{3}H]ouabain binding to intact lymphoblasts

The cells were harvested by centrifugation at 700 × *g* for 15 min, washed once by resuspension in 5.0 ml potassium-free Ringer solution, collected by centrifugation, and then resuspended in the Ringer solution before assay. The binding of [^{3}H]ouabain (in ten concentrations between 5 and 40 nM) to cells (typically $2 \cdot 10^5$ to $1 \cdot 10^6$ cells per tube) was measured after incubation in a total volume of 500 µl Ringer solution for 1.5 h at 37°C. At this time the binding of [^{3}H]ouabain was in equilibrium at all the concentrations used. Bound and unbound [^{3}H]ouabain were separated by centrifugation at 2000 × *g* for 5 min at 4°C. The cells were washed once by resuspension in 750 µl ice-cold Ringer solution and collected by centrifugation. The cell pellet was digested in 1.0 M NaOH (500 µl). Radioactivity was determined in an aliquot (400 µl) of the resulting solution. Non-specific binding of [^{3}H]-ouabain to the cells, defined using 0.1 mM non-radioactive ouabain, was always less than 1% of total binding. The K_d and B_{max} of ouabain binding to the cells was determined by analysis of the data using the method of Scatchard [15]. The results have been expressed as fmol ouabain bound per 10^6 cells (B_{max}) and nM (K_d).

Measurement of rubidium uptake by intact lymphoblasts

Cells were harvested and washed as described above. The uptake of rubidium was measured at six concentrations of non-radioactive rubidium between 0.6 and 6.0 mM containing a tracer concentration of ^{86}Rb (1.0 µM). Non-specific uptake of rubidium, defined using 0.1 mM ouabain was usually about 30% of total uptake. Cells (typically between $4 \cdot 10^5$ and $1.5 \cdot 10^6$ cells per tube) were incubated with rubidium at 37°C for 3 min in a total volume of 500 µl potassium-free Ringer solution. The uptake of rubidium into the cells was linear with respect to time for 5 min at all of the concentrations of rubidium used. Uptake was terminated by cooling the mixture to 0°C in an ice-water bath. Intracellular and extracellular rubidium were separated in the same way as for the separation of bound and unbound ouabain. The K_m and V_{max} of rubidium uptake into the cells were determined by analysis of the data using the method described by Garay and Garrahan [16], assuming two external binding sites for rubidium. The results have been expressed as nmol of rubidium per 10^6 cells per min (V_{max}) and mM (K_m).

Measurement of [^{3}H]thymidine incorporation into DNA

The rate of DNA synthesis in the lymphoblasts was estimated by measuring the rate of incorporation of [^{3}H]thymidine into the acid insoluble cellular fraction. Cells in RPM1–1640 (450 µl, typically $2 \cdot 10^5$ to $1 \cdot 10^6$ cells) and [^{3}H]thymidine (50 µl, 10 $\mu Ci \cdot ml^{-1}$ in saline) were incubated for 2 h at 37°C. The cells were transferred, washing with potassium-free Ringer, to Whatman GF/C glass fibre filters on a Millipore filter manifold. The cells were washed three times with 5 ml ice-cold isotonic phosphate-buffered saline, three times with 5 ml of ice-cold perchloric acid (1.5%), and once with 10 ml ethanol. The radioactivity on the filters was determined and the results have been expressed as fmol of thymidine incorporated per 10^6 cells per h.

Determination of the activity of γ-glutamyl transferase

Cells (typically $2 \cdot 10^6$) in 100 µl potassium-free Ringer were added to 2.0 ml 0.05 M Tris-HCl buffer (pH 8.2) containing 4 mM L-γ-glutamyl-*p*-nitroanilide, 50 mM glycylglycine, and 10 mM $MgCl_2$. The tubes were incubated for 1 h at 37°C. The tubes were centrifuged at 2000 × *g* for 5 min at 20°C and the absorbance of the solutions at 405 nm was recorded. The results have been expressed as a percentage of the activity recorded using cells maintained at a $[K]_0$ of 5.0 mM.

Determination of the intracellular water volume and of cell volume

The volume of intracellular water was determined using the method of Lichtstein et al. [17]. Cells (typically $(1–2) \cdot 10^6$ cells in 450 µl Ringer solution) were exposed to a mixture of ^3H_2O and [^{14}C]sorbitol (50 µl, containing approx. 10^5 dpm of each compound) for 2 min at 4°C. The cells were collected by centrifugation at 2000 × *g* for 5 min at 4°C, dissolved in 500 µl 1.0 M NaOH and the radioactivity was determined in 400 µl of the resulting solution. The total volume of water in the cell pellet was calculated from the ^3H_2O content of the cell pellet, and the extracellular volume was calculated from the [^{14}C]sorbitol content of the cell pellet. The difference between these two volumes represents the intracellular water volume.

Cell volumes were measured using a Coulter Counter.

Determination of the intracellular contents and concentrations of sodium and potassium

Cells, typically $5 \cdot 10^6$ in 1.0 ml Ringer solution, were added to 10 ml ice cold isotonic $MgCl_2$ and centrifuged

at 2000 × *g* for 5 min at 4°C. The cell pellet was lysed in 5.0 ml water. Na and K were determined by atomic absorption spectrophotometry using a Perkin-Elmer Model 2380 atomic absorption spectrophotometer using emission detection.

Cell sodium and potassium concentrations were calculated by dividing cell potassium and sodium contents either by cell water volume or by the cell volume.

Statistical analyses

The data are presented as mean (±S.E.). Statistical comparisons have been made by unpaired, two-tailed Student's *t*-tests.

Results

Ouabain binding and rubidium influx characteristics of BM1A lymphoblasts

When BM1A lymphoblasts were maintained in RPMI-1640 medium containing 10% fetal calf serum and 5 mM KCl the intracellular concentrations of sodium and potassium were 29 and 181 mmol per litre cell water, respectively. Under these conditions the ouabain binding capacity of the cells (B_{max}) was 1580(±50) fmol per 10^6 cells ($n = 25$). This was equivalent to approx. 950000 Na/K-ATPase sites per cell, assuming that one ouabain molecule binds to one Na/K-ATPase site. The apparent dissociation constant of ouabain for the Na/K-ATPase (K_d) was 14.0(±0.8) nM ($n = 66$). The rubidium uptake capacity of the cells (V_{max}) was 3.54(±0.17) nmol per 10^6 cells per min ($n = 13$). This was equivalent to a rate of transport of rubidium across the cell membrane of $2.13 \cdot 10^9$ molecules per cell per min. Hence, the turnover number was approx. 2240 molecules of rubidium per Na/K-ATPase site per min. The transport K_m of rubidium by the Na/K-ATPase was 0.48(±0.02) mM ($n = 24$). This value is similar to the K_i for the inhibition of rubidium uptake in these cells by potassium, which is a competitive inhibitor ($K_i = 0.52(\pm 0.08)$ mM).

Maintenance of BM1A cells in RPMI-1640 supplemented with 10% dialysed fetal calf serum, as opposed to undialysed fetal calf serum, did not affect cell growth, ouabain binding, or rubidium uptake (data not shown).

The effects of a decreased extracellular concentration of potassium on BM1A lymphoblasts

Fig. 1 shows the effect on the B_{max} of ouabain binding and the V_{max} of rubidium uptake of exposure of the cells to lowered extracellular concentrations of potassium (0.3 to 5.0 mM) for both 24 h (left) and 72 h (right). At 24 h the V_{max} of rubidium uptake was increased, the maximum change of 42% occurring at an extracellular potassium concentration of 0.4 mM (significantly different from 5.0 mM KCl, $P < 0.05$). In contrast, the B_{max} of ouabain binding was unchanged at

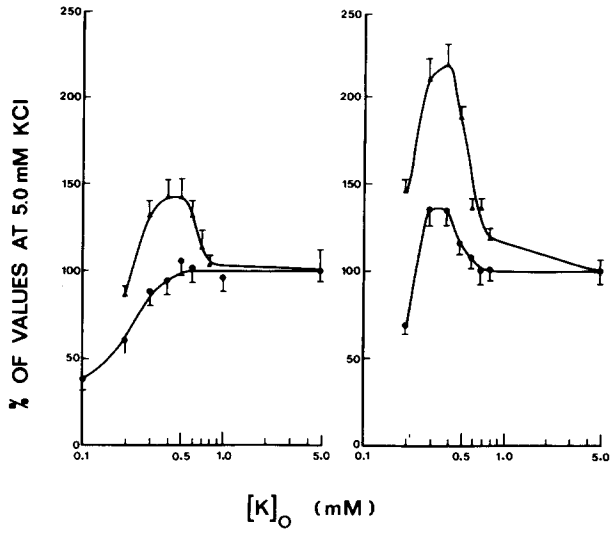

Fig. 1. The effects of a low $[K]_0$ for 24 h (left) and 72 h (right) on the B_{max} of [^{3}H]ouabain binding (●) and the V_{max} of ^{86}R6 uptake (▲) by BMIA lymphoblasts. Each point represents a mean (S.E.) of three separate experiments.

these concentrations. This means that there was an increase in the turnover number of rubidium from 2760(±370) to 4130(±380) molecules per site per min. At a $[K]_0$ below 0.3 mM both V_{max} and B_{max} decreased.

In marked contrast, after exposure of the cells to low $[K]_0$ (0.3 to 0.5 mM) for 72 h both the V_{max} and the B_{max} increased. The maximum changes were 118% and 35%, respectively (significantly different from 5.0 mM KCl, $P < 0.002$ and $P < 0.01$, respectively), and occurred at a $[K]_0$ of 0.4 mM. This corresponds to an increase in the turnover number of rubidium from 1900(±180) to 3070(±270) molecules per site per min. At $[K]_0$ below 0.4 mM both V_{max} and B_{max} decreased in a manner similar to that seen after incubation for 24 h. The K_d of ouabain binding was not affected by the low $[K]_0$ (data not shown). However, the K_m of rubidium uptake increased after incubation of the cells for 72 h at a low $[K]_0$ (Fig. 2). No change in K_m was observed

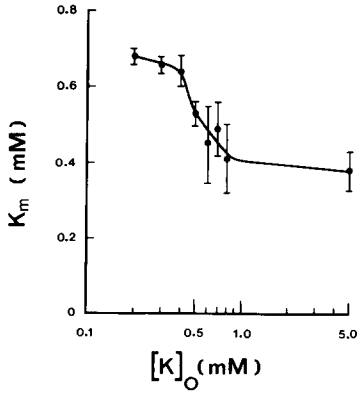

Fig. 2. The effects of a low $[K]_0$ for 72 h on the K_m of ^{86}R6 uptake into BMIA lymphoblasts. Each point represents a mean (S.E.) of three separate experiments.

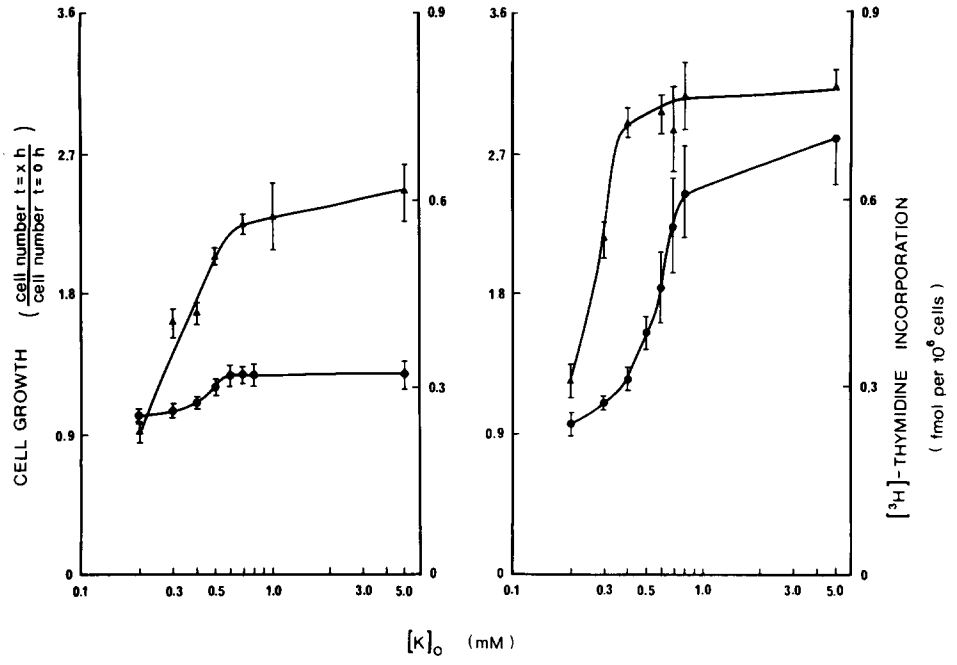

Fig. 3. The effects of a low $[K]_0$ for 24 h (left) and 72 h (right) on cell growth (●) and [^{3}H]thymidine incorporation into acid-insoluble counts (▲). Each point represents a mean (S.E.) of three separate experiments.

after incubation for 24 h at a low $[K]_0$ (data not shown).

Fig. 3 shows the effect of exposure to low $[K]_0$ for 24 h (left) and 72 h (right) on the ability of BM1A cells to synthesize DNA and to divide. The inhibition of the incorporation of [^{3}H]thymidine into DNA was similar after exposure to low $[K]_0$ for 24 h and 72 h. The lowest value of $[K]_0$ which permitted DNA synthesis to proceed at control levels was 0.5 mM. However, this potassium concentration markedly affected the growth of the cells, their ability to proliferate being severely inhibited when they were exposed to a $[K]_0$ below 0.8 mM for 72 h.

The specificity of the effects of a low $[K]_0$ on the Na/K-ATPase was examined by measuring the activities of γ-glutamyl transferase and 5′-nucleotidase, two

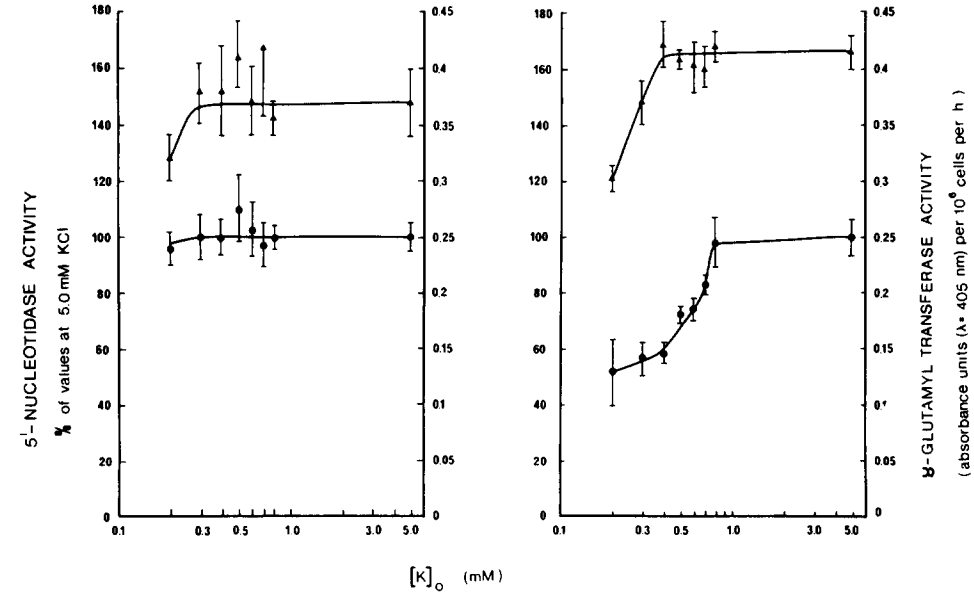

Fig. 4. The effects of a low $[K]_0$ 24 h (left) and 72 h (right) on the activities of 5′-nucleotidase (●) and γ-glutamyl transferase (▲) in BM1A lymphoblasts. Each point represents a mean of two separate experiments.

other integral plasma membrane enzymes, following exposure to a low $[K]_0$ (Fig. 4) for 24 h (left) and 72 h (right). No increase in the activity of either enzyme was observed after exposure to low $[K]_0$ for both 24 h and 72 h. Very low $[K]_0$ caused decreases in the activities of both enzymes.

The effect of a 24 h exposure to low $[K]_0$ on the intracellular concentration of sodium and potassium (mmol/l cell volume) is shown in Fig. 5. When $[K]_0$ was less than 0.7 mM, the $[Na]_i$ increased but $[K]_i$ was unchanged; the value of $[Na]_i$ at a $[K]_0$ of 0.3 mM was four times its value at a $[K]_0$ of 5 mM. Only when $[K]_0$ fell to 0.2 mM, did the $[K]_i$ fall.

Exposure to a low $[K]_0$ caused a linear reduction in cell volume (data not shown). This reduction in cell volume was proportional to the change in intracellular potassium content at extracellular potassium concentrations down to 0.3 mM, which is why intracellular potassium concentrations were unaffected by a reduction in extracellular potassium concentrations. However, the increase in intracellular sodium content was proportionally greater than the concomitant fall in cell volume, so that intracellular sodium concentrations rose with reduced extracellular potassium concentrations. The changes in cell volume which occurred in response to decreased $[K]_0$ were the same at 24 h as at 72 h.

The effects of monensin on BM1A lymphoblasts

The role of $[Na]_i$ in triggering the increase in both the number and activity of Na/K-ATPase sites in BM1A cells was investigated using monensin, an ionophore which selectively increases intracellular sodium. Fig. 6 shows the effect of a 2 h exposure of BM1A cells to monensin on intracellular sodium. At monensin concentrations greater than 0.43 μM the intracellular sodium was significantly increased compared with controls. Under these conditions intracellular potassium was unaffected by monensin ($[K]_i = 93.0(\pm 2.7)$ nmol per 10^6 cells). Fig. 6 also shows the effect of a 10 min

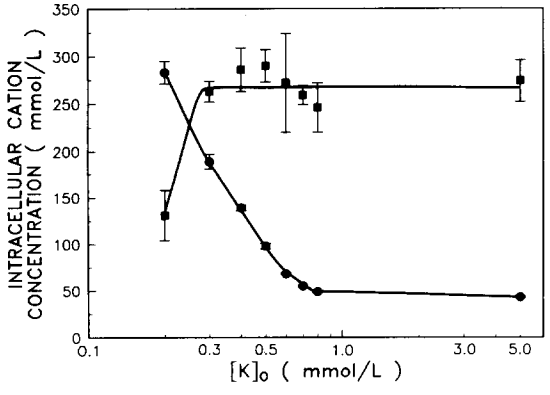

Fig. 5. The effects of a low $[K]_0$ for 24 h on the intracellular concentrations of potassium (■) and sodium (●) in BM1A lymphoblasts.

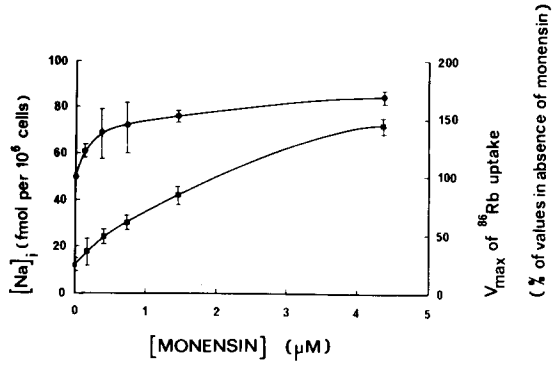

Fig. 6. The effects of exposure of BM1A lymphoblasts to monensin for 2 h or intracellular sodium levels (■) and to monensin for 10 min on the V_{max} of ^{86}R6 uptake (●). Each point represents a mean of two separate experiments.

exposure of BM1A cells to monensin on the V_{max} of rubidium uptake. Monensin increased the V_{max} of rubidium uptake to about 170% of the value measured in the absence of monensin ($P < 0.01$). The increase in V_{max} was maximum at monensin concentrations greater than 1.44 μM. Under these conditions of short-term incubation the B_{max} of ouabain binding was unaffected by monensin (data not shown).

In marked contrast, when BM1A cells were exposed to 11.5 nM monensin for 72 h the B_{max} of ouabain binding was increased to about 128% of the value measured in the absence of monensin. This increase in the B_{max} was accompanied by an increase in intracellular sodium (8.37($\pm$0.84) nmol per 10^6 cells in control cells and 12.03($\pm$0.44) nmol per 10^6 cells in monensin-treated cells). The intracellular potassium was unaffected by this treatment (123 nmol per 10^6 cells in control cells and 123.4 nmol per 10^6 cells in monensin-treated cells). Cell death resulted when cells were exposed to concentrations of monensin greater than 30 nM for 72 h.

Discussion

Ouabain binding and rubidium influx characteristics of BM1A lymphoblasts

The number of ouabain binding sites (and hence, by inference, of Na/K-ATPase molecules) in the cell membrane of BM1A lymphoblasts is approx. 30-times that found in normal lymphocytes (950000 vs. 30000, respectively) [3,18,19,20], but is similar to the value of $7.2 \cdot 10^5$ sites per cell found in HeLa cells [21]. It is possible that the increased metabolic activity of the lymphoblasts requires a greatly increased Na/K-ATPase activity, but the mechanism whereby the number of Na/K-ATPase sites increases after transformation with EB virus is not known. The mitogen phytohaemagglutinin can also increase the number of Na/K-ATPase molecules in human lymphocytes, but the effect is very

small, the increase being only 84% over several days [22]. Similar results have been obtained following mitogenic activation of lymphocytes with concanavalin A [23]. The apparent dissociation constant of ouabain for the Na/K-ATPase of BM1A cells ($K_d = 14$ nM) is seven times greater than the value of 2 nM reported for normal lymphocytes [18]. The significance of this difference is not known. However, the turnover number of the Na/K-ATPase sites (2240 ions per site per min) is similar to that which we have found in the lymphocytes of healthy subjects (about 4500 ions per site per min [24] and to that reported in HeLa cells [6].

It may be that even higher turnover rates are possible under some conditions in these cells, since higher values of V_{max} can be achieved if the intracellular sodium and extracellular potassium concentrations are manipulated appropriately [25]. However, since we have measured ^{86}Rb influx at the same extracellular concentration of potassium in all cases, the only factor which changed was the intracellular sodium concentration, and that would explain the increase in the apparent V_{max} at 24 h described above. The fact that there was a further increase in V_{max} at 72 h in association with a proportional increase in B_{max} at that time, but without any further change in intracellular sodium concentration, suggests that the increase in V_{max} which occurred at between 24 h and 72 h was due to a true increase in the number of Na/K pumps in the cell membrane.

The effects of a decreased extracellular concentration of potassium on BM1A lymphoblasts

Exposure of BM1A cells to decreased $[K]_0$ for 24 h caused an increase in the V_{max} of the rate of inward transport of rubidium by the Na/K-ATPase (Fig. 1). This increase occurred in the presence of an increase in the intracellular sodium concentration, as one would expect from the normal functioning of the Na/K pump [16], and this would be expected to tend to counteract the initial change in $[Na]_i$ caused by reducing $[K]_0$. This effect has previously been described in giant squid axons and human erythrocytes [16]. Under these conditions, no change was observed in the number of Na/K-ATPase molecules in the cell membrane, as measured by ouabain binding. This response to a decrease in $[K]_0$ was not a generalized phenomenon, since the activities of neither γ-glutamyl transferase nor 5′-nucleotidase were increased by this treatment (Fig. 3). However, the ability of the cells to increase the rate of ion transport by the Na/K-ATPase in response to this stress was limited, because a $[K]_0$ of less than 0.5 mM caused a decrease in both B_{max} and V_{max} during the 24 h period.

In contrast, when BM1A cells were exposed to decreased $[K]_0$ for 72 h both the B_{max} and the V_{max} increased, by 35% and 118%, respectively, at a $[K]_0$ of 0.4 mM (Fig. 1). In this case sufficient time had elapsed from the onset of the stress to enable the cells to synthesize and insert new Na/K-ATPase molecules into the cell membrane. Thus, although the turnover number after 72 h at a $[K]_0$ of 0.4 mM (3070 min^{-1}) was higher than at a $[K]_0$ of 5.0 mM (1900 min^{-1}) it was lower than that after 24 h (4130 min^{-1}) when no increase in enzyme number was evident.

This result confirms the observations that others have made in HeLa cells [6] and MDCK cells [7] in vitro and in guinea-pig erythrocytes in vivo [8]. However, discordant results have been reported in both the skeletal muscle of man, rats, and guinea-pigs, in which hypokalaemia reduces the number of Na/K pumps [8,11,12], and in the cardiac muscle of guinea-pigs, in which hypokalaemia has no effect [8]. A study of the effects of hypokalaemia induced by a low potassium diet in guinea-pigs, with simultaneous measurement of ouabain binding sites in erythrocytes, skeletal muscle, and cardiac muscle [8] has shown that these differences are not likely to be due to technical differences between laboratories, but to true differences between the responses of different tissues to the stimulus of hypokalaemia, although it is not clear what the nature of such differences might be.

The increase in intracellular sodium concentration which occurs during exposure of cells to a low $[K]_0$ has been proposed as the trigger for an increase in the number of Na/K pumps in cells in which this effect occurs [6,7]. However, the mechanism whereby the increase in sodium concentration does this is not known. Exposure of BM1A lymphoblasts to a low $[K]_0$ also caused a significant reduction in cell volume. Cell shrinkage is known to result in increased activity of the Na/H antiport, which acts as a regulatory mechanism counteracting the reduction in volume [26]. We have elsewhere shown that an increase in Na/H antiport activity, produced by exposing normal lymphocytes to lithium for 72 h, is associated with an increase in Na/K pump numbers and an increased intracellular pH; we have also shown that the increase in Na/K pump numbers caused by lithium can be prevented by the addition of the Na/H antiport inhibitor dimethylamiloride [27,28]. Thus, we believe that an increase in intracellular sodium concentration may trigger an increase in Na/K pump numbers by causing an increase in Na/K pump protein synthesis in response to an increase in intracellular pH, which in turn occurs via increased Na/H antiport activity [29].

At extracellular concentrations of potassium associated with an increase in the number and activity of the Na/K-ATPase sites in the cell membrane, there was either no change or a decrease in cell growth and DNA synthesis. Thus, the increases in V_{max} and B_{max} of rubidium uptake and ouabain binding occurred independently of changes in DNA synthesis and cell growth.

The values of $[K]_0$ which affected the Na/K-ATPase, DNA synthesis, cell growth, γ-glutamyl transferase and

5′-nucleotidase all differed from each other. DNA synthesis was relatively insensitive to changes in $[K]_0$, while cell growth was affected by relatively small alterations in $[K]_0$ (Fig. 3), probably reflecting the central involvement of potassium in the enzymes of protein synthesis [30]. The markedly differing sensitivities of γ-glutamyl transferase and 5′-nucleotidase to changes in $[K]_0$ (Fig. 4) may reflect changes in the turnover rates of these enzymes.

The effects of monensin on BM1A lymphoblasts

As mentioned above, it has been suggested that the increase in $[Na]_i$ which occurs in response to a decrease in $[K]_0$ may be responsible for the increase in the rate of ion transport by the Na/K-ATPase [6,7]. Under normal conditions when $[K]_0$ is 5 mM, $[Na]_i$ may be rate-limiting for the rate of ion transport by the Na/K-ATPase. As a consequence, any estimation of V_{max} would be compromised by the non-saturating concentration of $[Na]_i$. Hence, any intervention which increases $[Na]_i$ would also apparently increase the V_{max} of rubidium uptake. This assumption was further tested using the polyether monocarboxylic acid sodium ionophore monensin [31], which has been shown to increase the initial rate of uptake of $^{86}Rb^+$ into 3T3 mouse cells [32]. In the present study monensin increased intracellular sodium without affecting intracellular potassium (Fig. 6). It also increased both the initial rate of uptake of rubidium (data not shown) and its V_{max} by 70% (Fig. 6). This increase in V_{max} is similar to that achieved (42%, Fig. 1) under conditions which affected Na/K-ATPase activity without affecting enzyme numbers, ie incubation for 24 h at a $[K]_0$ of 0.4 mM. Given that monensin increased the activity of the Na/K-ATPase after a 10 min incubation, it should also increase the number of Na/K-ATPase molecules present in the cell membrane over a longer incubation period. This was confirmed by incubation of cells for 72 h in the presence of 11.5 nM monensin. Under these conditions intracellular sodium was increased by 42%, was unchanged, and the B_{max} of ouabain binding was increased by 28%.

Thus, it may be concluded that a decrease in $[K]_0$ causes an increase in $[Na]_i$ in BM1A cells. In the short term the cells are able to adapt to this stress by permitting the Na/K-ATPase to pump harder. In the longer term, however, this is insufficient to maintain transmembrane ion gradients, and as a consequence the number of Na/K-ATPase sites increases. This permits the maximum activity of the Na/K-ATPase to increase further. The value of $[Na]_i$ may be the trigger which causes the increase in the number of Na/K-ATPase sites in the membrane, since monensin, which affects intracellular sodium without affecting intracellular potassium, is capable of eliciting both the short-term and long-term effects of low $[K]_0$ in the BM1A cell line. This is in agreement with earlier studies in the HeLa cell line, which suggested that $[Na]_i$ regulated the cellular response to a low $[K]_0$ [6,21].

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