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Expression of mRNAs encoding the α1 and the β1 subunits of Na^+,K^+-ATPase in human lymphocytes activated with phytohaemagglutinine

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Increase in Na^+,K^+-ATPase mRNAs was detected in activated lymphocytes by the RT-PCR method. α1 subunit mRNA gradualy increased with time and by 36 h was 2.4 times higher than at the start. Increase in the β1 mRNA was transient reaching a maximum in the 8 h probe and declining to the initial level in the 24 and 36 h probes. The elevation of Na^+,K^+-ATPase mRNAs does not underlie a cycloheximide-inhibited increase in cation pumping peculiar to the prereplicative period as can be judged from the fact that Act D fails to eliminate PHA-induced enhancement of pump fluxes.

Cell proliferation; Lymphocyte activation; Human gene expression; Na^+,K^+-ATPase mRNA; Potassium flux; Actinomycin D

1. INTRODUCTION

Human lymphocytes activated with phytohaemagglutinin (PHA) have long been used as a model for studies of the transition of animal cell from quiescence to proliferation. As in other cells, the onset of lymphocyte proliferation is accompanied by a series of changes in monovalent ion transport across the plasma membrane [1–5]. In the experiments on human lymphocytes activated with PHA we have found that a long-term increase in Na^+,K^+-ATPase ion transport at the prereplicative stage of blastogenesis was abolished under the inhibition of protein synthesis with cycloheximide [4]. It has been shown in several species of cells that the generation of new Na^+,K^+-pumps can be mediated by an increase in Na^+,K^+-ATPase mRNAs [6–10]. However, no data are available on the expression of Na^+,K^+-ATPase mRNAs in lymphoid cells nor in any other cells which progress from dormancy to proliferation in accordance with their natural function.

2. MATERIALS AND METHODS

Peripheral blood lymphocytes were isolated from fresh venous blood of healthy donors by Ficoll–Paque gradient centrifugation. Cells were washed three times with Ca-free Hanks' balanced salt solution and resuspended in RPMI-1640 medium (Flow) supplemented with glutamine (2 mM) and heat-inactivated human AB 1V Rh(+) serum and without any antibiotics. For experiments the cell suspension was made up to a concentration of $(1.5–2) \times 10^6$ cells/ml, placed into small vials (2 ml/vial) and PHA-M (Calbiochem) was added to a final concentration of 20 μg/ml. Before the end of the experiment cells were pulsed if necessary with [^{3}H]thymidine, [^{14}C]leucine or [^{3}H]uridine (Isotope, Russia) for 2 h and by Rb^+ (2.5 mM) with or without ouabain (Sigma, 0.1 mM) for 30 min. Thereafter cell suspensions were transfered into Eppendorf tubes, pelleted by centrifugation at $3000 \times g$ for 3 min, rinsed five times with cold isotonic $MgCl_2$ without dispersion and treated with 1 ml 5% trichloroacetic acid (TCA, Reachim, Russia). TCA extracts were analyzed for Na^+, K^+ and Rb^+ by flame emission using a Perkin-Elmer spectrophotometer AA 306. TCA precipitates were dissolved in 0.1 NaOH and analyzed for protein and radiolabels.

Total cellular RNA was isolated by the method of Birnboim [11]. Reverse transcription and polymerase chain reaction (RT-PCR) were carried out as described by Akopyanz et al. [12]. (RT-PCR procedure was performed by E.O. Marzen under the guidance of N.E. Broude and G.S. Monastyrskaya in the Lab. of Human Genes headed by E.D. Sverdlov, Institute of Bioorganic Chemistry, Moscow. The authors greatly appreciate their collaboration). Densitometry was done by a computer-controlled image analysis system ($512 \times 512 \times 8$ frame grabber + IBM PC + IZOT 1403 software, Bulgarian).

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3. RESULTS AND DISCUSSION

The bands identified as α1 and β1 mRNA fragments were detected in all samples obtained from both quiescent and activated lymphocytes (Fig. 1). The intensity of the bands, i.e. relative abundance of the α1 and β1 subunit mRNAs, at different stages of lymphocyte activation appeared to be different (Fig. 2). α1 mRNA gradually increased with time and by 36 h was 2.4 times higher than at the start. By contrast, an increase in the amount of β1 mRNA was transient reaching a maximum in the 8 h probe and declining to the initial level in the 24 and 36 h probes. Only small changes, if any, were observed for human β cytoplasmic actin mRNA.

The sodium pump has traditionally been thought to

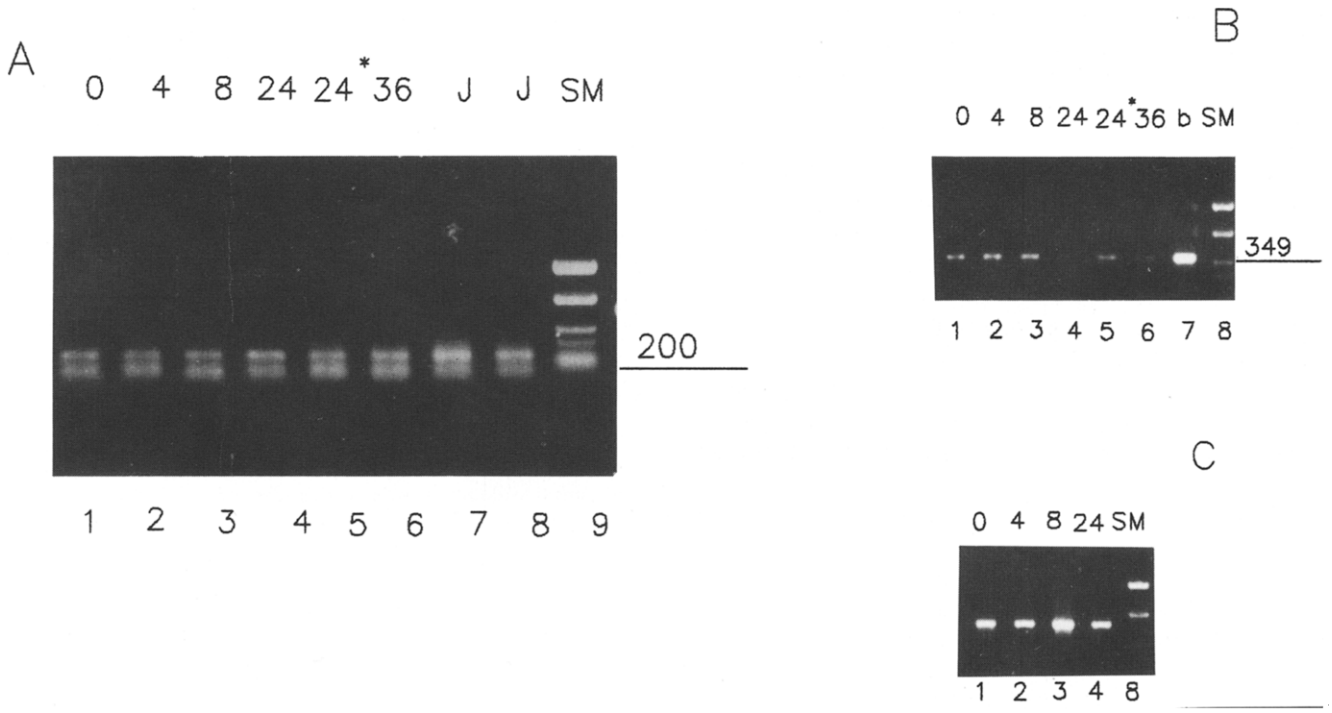
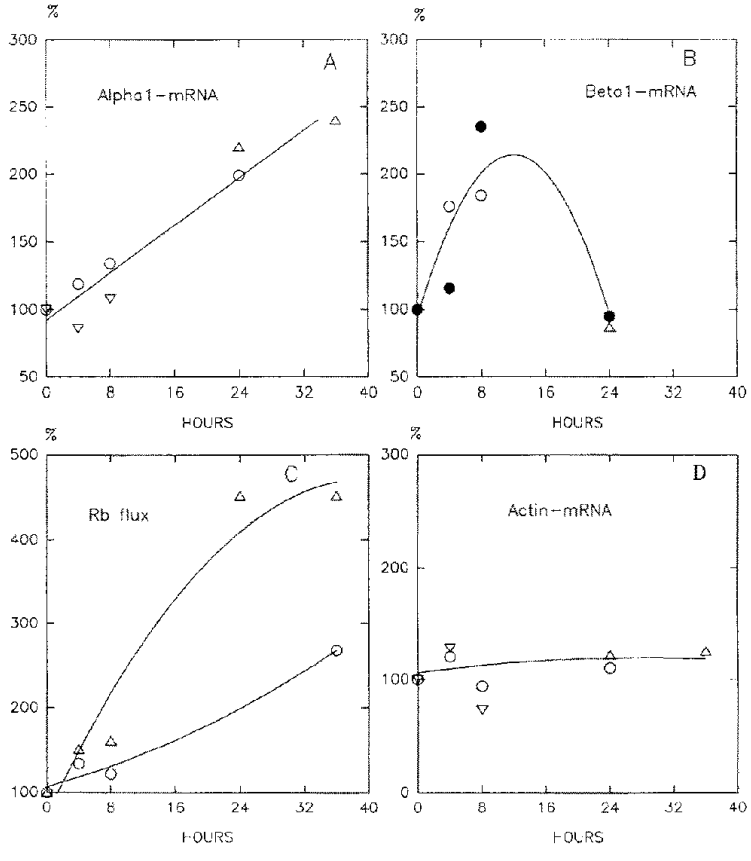


Fig. 1. RT-PCR analysis of Na,K-ATPase mRNAs abundance in quiescent and activated human lymphocytes. A. RT-PCR products after cDNA amplification with primers specific to $\alpha 1$ subunit mRNA. Lane 1, quiescent lymphocytes (control); lanes 2–6, activated lymphocytes; lane 2, 4 h; lane 3, 8 h; lane 4, 24 h; lanes 5, 6 (other donor), 24 and 36 h after activation, respectively; lanes 7 and 8, T lymphoblastoid cell line Jurkat cultivated with (8) or without (7) ouabain $2 \cdot 10^{-8}$ M for 24 h; lane 9, size marker pUC19/Sau3A. B. RT-PCR products after cDNA amplification with primers specific to $\beta 1$ subunit mRNA. Lane 1, resting lymphocytes (control); lanes 2–6, activated lymphocytes; lane 2, 4 h, lane 3, 8 h, lanes 4, 5, 24 h; lane 6, 36 h after activation; lane 5, 6, other donor; lane 7, brain; lane 8, size marker pUC19/Sau3A. C. Repeated electrophoresis of RT-PCR products reported in B, same symbols as in B.



serve fundamental housekeeping functions in the cell. So, it can be expected that the number of sodium pumps during cell growth will increase in parallel with all other cell body components. Accordingly, the mRNAs encoding constituent proteins should proportionally increase. An increase in the abundance of Na^+,K^+-ATPase mRNAs in our experiments implies that the ratio between total and Na^+,K^+-ATPase mRNAs does not hold. In view of this we might say that Na^+,K^+-ATPase genes behave as growth- or proliferation- or differentiation-related genes rather than just housekeeping genes. Elevation of $\alpha 1$ and $\beta 1$ subunit mRNAs concomitant to an increase in proliferation of cells in culture was reported for K^+- depleted [10] and serum-depleted-restored [6,8] liver cell lines as well as for the regenerating rat liver [8].

An increase in cell Na^+ is thought to be the factor triggering the extra-expression of Na^+,K^+-ATPase mRNAs under certain conditions [7,9,10,13]. In our measurements cell Na^+ in PHA-activated lymphocytes by 2 h was higher than at the start by about 50% and then remained practically at the same level. Mean values for 0.5, 2, 5, 24 and 48 h were 160 ± 11 (S.E.M., n = 24), 220 ± 12 (n = 23), 220 ± 13 (n = 24), 240 ± 17 (n = 24) and 250 ± 21 (n = 18) μmol/g protein, respectively. The changes are rather moderate as compared with those presumed in cells treated for a long time with ouabain or veratridine in the studies of 'ion-dependent' Na^+,K^+-ATPase upregulation [13]. From this we can conclude that the increase in Na^+,K^+-ATPase mRNAs in activated lymphocytes is caused either by a relatively small change in the intracellular sodium concentration or by some mechanism. Such a mechanism has recently been demonstrated for coordinate regulation of the A system for amino acid transport and Na^+,K^+-ATPase $\alpha 1$ subunit mRNA in the mutant CHO cells [14]. In this case upregulation of the $\alpha 1$ subunit mRNA occurred along with a decrease in intracellular Na^+ and was mediated by regulatory gene R 1.

It is known that the 1:1 ratio between α and β polypeptides in the Na^+,K^+-ATPase complex is required for the functional activity of the sodium pump to develop [15]. Different time courses for the expression of $\alpha 1$ and $\beta 1$ mRNAs in activated lymphocytes indicate that the correct subunit stoichiometry is achieved at the posttranscriptional stages of the generation of new pumps. Similar asynchrony in the expression of

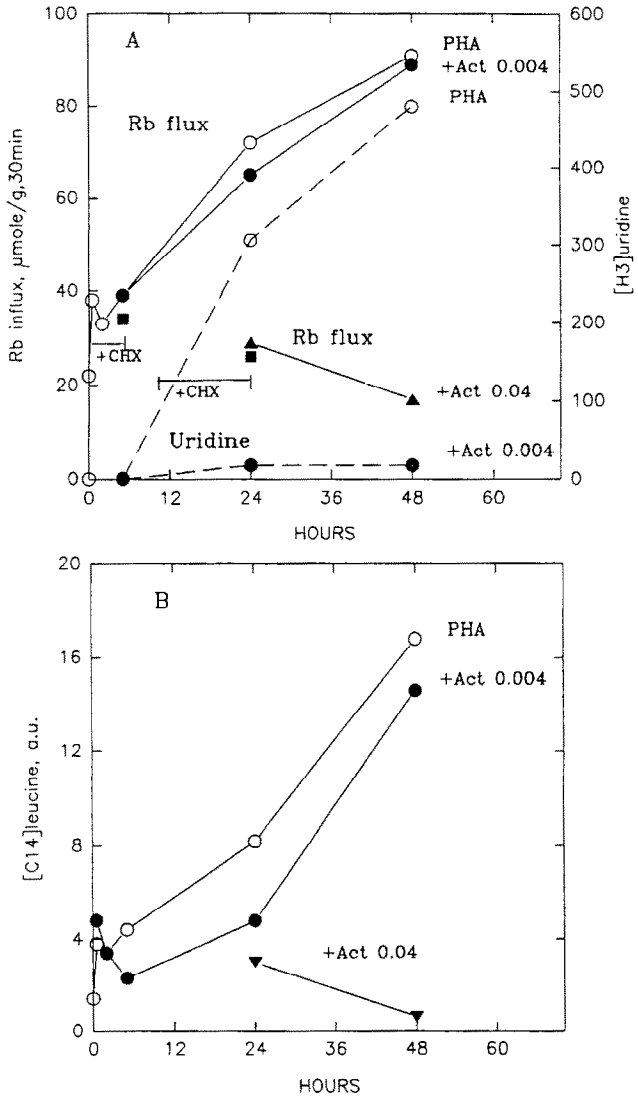


Fig. 3. Long-term increase in Na,K-ATPase-mediated Rb influx and [^{3}H]uridine incorporation in PHA-activated human lymphocytes under the inhibition of RNA synthesis with Act D. A. Solid lines, ouabain-sensitive Rb influx (μmol/g protein, 30 min); dashed lines, [^{3}H]uridine incorporation (a.u.). Open circles, control (no inhibitor); closed symbols, with Act D (circles, 0.004 μg/ml; triangles, 0.04 μg/ml) or with cycloheximide (CHX), squares. To diminish the indirect effect of CHX on cation transport, i.e. the effect caused by inhibition of synthesis of proteins other than Na^+,K^+-ATPase with life time shorter than Na^+,K^+-ATPase, it was administered during the periods 0–5 h or 8–24 h of lymphocyte activation. Act D was introduced into the medium together with PHA. B. [^{14}C]leucine incorporation: the same symbols.

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Fig. 2. Changes in Na,K-ATPase mRNAs abundance in PHA-activated human lymphocytes. A. $\alpha 1$ subunit mRNA. B.$\beta 1$ subunit mRNA. C. Na, K-ATPase-mediated Rb influx. D. β-actin mRNA. The results obtained on lymphocytes from one and the same donor are shown by the same symbols. Closed and open circles indicate two independent electroporeses of the same RT-PCR products. The values for unstimulated lymphocytes are taken as reference.

Na^+,K^+-ATPase subunit mRNAs has been described in veratridine-treated chicken myocytes [9].

It seemed of interest to examine the long-term ionic events in activated lymphocytes under the inhibition of transcription by actinomycin D (Act D). It has been found that at a concentration of 0.004 μg/ml sufficient for the suppression of [^{3}H]uridine incorporation into

TCA-precipitable cell material, the long-term increase in K^+ pump fluxes is not abolished (Fig. 3). However, after the application of cycloheximide (5 μg/ml), this increase is fully abolished. These data suggest that the newly synthesized pump polypeptides, responsible for an increase in ion fluxes via the pump pathway at a later stage of lymphoblastogenesis, are translated from the preexisting Na^+,K^+-ATPase mRNAs. Act D at higher concentrations (0.04 μg/ml) diminished the ouabain-sensitive Rb^+ influx by about 66% at 24 h and by 81% at 48 h of lymphocyte activation (Fig. 3). However, this inhibitory effect was accompanied by a significant decrease in total [^{14}C]leucine incorporation, and therefore, should be considered to be indirect. Thus, it can be concluded that the elevation of Na^+,K^+-ATPase mRNAs at a later stage of lymphocyte blastogenesis is of no importance in the increase of cation pumping peculiar to prereplicative period. Whether or not it is necessary for the subsequent stages of cell cycle remains so far unclear.

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