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Long-term enhancement of Na,K-ATPase pump during blasttransformation of human lymphocytes is controlled first by translational, then by transcriptional mechanisms

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Abstract The transition of phytohemagglutinin-activated human lymphocytes from resting state to proliferation is accompanied by a long-term increase in ouabain-sensitive Rb(K) influx which is closely related to a cyclosporin A-sensitive step of G0/G1/S progression. At least two distinct phases of the up-regulation of cation pump has been revealed: the initial stage (5–20 h) which is cycloheximide-inhibitable and actinomycin D (α-amanitin)-unaffected, and the later stage (after 20 h) which is cycloheximide- and actinomycin D (α-amanitin)-inhibitable. Thus, the enhanced Na,K-ATPase pump during the cell progression from quiescence to proliferation is controlled both at translational and transcriptional levels.

Key words: Lymphocyte activation; Na,K-ATPase pump; Cyclosporin A; Cycloheximide; Actinomycin D; α-Amanitin

1. Introduction

Potassium influx via the Na,K-ATPase pump has been shown to increase significantly in animal cells stimulated from quiescence to proliferation: a rapid elevation of the ion pump flux in response to mitogens is followed by a long-term increase in the pump activity in the course of the G0/G1/S transition [1–5]. The initial activation of the Na,K-ATPase pump has been considered to result from the elevation of intracellular Na concentration (Na_i) due to an enhanced Na/H exchange [2,6]. The mechanisms controlling the long-term elevation of the Na,K-ATPase pump flux as well as the nature of the link between the mitogenic reaction and the late ionic changes are poorly understood.

Our recent studies on human lymphocytes activated by phytohemagglutinin (PHA) have shown that the long-term increase in pump-mediated K fluxes is limited to the prereplicative stage and coincides in time with the blasttransformation of lymphocytes [7]. It has been suggested that the enhanced cation pumping is an essential element of the whole program of cell transition from quiescence to proliferation. The goal of the present study was to find out whether the functional expression of Na,K-ATPase during the G0/G1/S progression is controlled via a genomic pathway.

2. Materials and methods

Peripheral blood lymphocytes were isolated from the fresh venous blood of healthy donors at Ficoll-verografin gradient centrifugation (1.077 g/ml, 400 × *g*). Cells were washed three times with Ca-free Hanks' salt solution and resuspended in RPMI-1640 medium (Flow) supplemented with glutamine (2 mM) and heat-inactivated AB IV Rh(+) serum, without any antibiotics. The cell suspension was made up to concentration of (1.5–2) × 10^6 cells/ml, placed into small vials (2 ml/vial) and PHA-M (Calbiochem) was added to a final concentration 20 μg/ml. Thereafter, the lymphocyte cultures were incubated at 37°C in a humidified atmosphere containing CO_2. At the appropriate time points throughout the activation experiment, RbCl (2.5 mM) with or without ouabain (0.1 mM, Sigma) as well as [^{14}C]leucine, [^{3}H]uridine and [^{3}H]thymidine (Isotope, Russia) were added to cultures for 30 min. At the end of the incubation time, cell suspensions were transferred into Eppendorf tubes and centrifuged once at 3,000 × *g* for 3 min. The cell pellets were rinsed five times with cold isotonic $MgCl_2$ without dispersion and treated with 1 ml of 1% trichloracetic acid (TCA). TCA extracts were analyzed for Rb, Na and K by flame emission using Perkin-Elmer spectrophotometer AA 306. TCA precipitates were dissolved in 0.1 N NaOH and analyzed for protein by Lowry procedure and for radiolabels by liquid scintillation counter (Beckman LS 8100). The cell cation content and the rates of radiolabel incorporation were calculated per g cell protein. The pump-mediated Rb influx was determined as the ouabain-inhibitable Rb uptake. Lymphocytes from one donor (up to 400 × 10^6 cells) were used in each experiment. Three or five cultures were used for each time point of the activation experiment.

The enlargement of lymphocytes after PHA stimulation was estimated by analyzing the volume distribution profile of fixed lymphocyte preparations using scanning microscope Magiscan-2 (Joice-Loebl).

3. Results and discussion

As shown in Fig. 1A, in PHA-stimulated human lymphocytes, an initial, within the first 2 h, twofold increase in the ouabain-sensitive Rb influx is followed by a plateau between 8 and 16 h, after which the Rb influx increases to a maximum value by 48 h and then declines. The comparison of the time course of Rb flux changes and macromolecular synthesis in activated lymphocytes shows that the late elevation of ouabain-sensitive Rb influx accompanies the transit to DNA synthesis and is parallel with the enlargement of cells (Fig. 1A and B).

Cyclosporin A (CsA) blocks transcription of interleukin-2 (IL-2), thus preventing the cell cycle progression of human lymphocytes [8]. As shown in Fig. 1D, in the presence of CsA (1 μg/ml) the enlargement of PHA-activated lymphocytes as well as the DNA synthesis are significantly suppressed. Under the same conditions the initial increase in ouabain-sensitive Rb influx (up to 24 h) is unaffected by CsA; however, between 24 and 48 h the flux elevation is abolished (Fig. 1C). It is of interest that CsA does not affect the time course of ouabain-resistant Rb influx (Fig. 1C). Thus, the long-term up-regulation of the Na,K-ATPase pump is closely associated with the CsA-sensitive and IL-2-dependent step of the lymphocyte activation. These findings suggest a close relationship between the enhanced cation pumping and the initiation of DNA synthesis.

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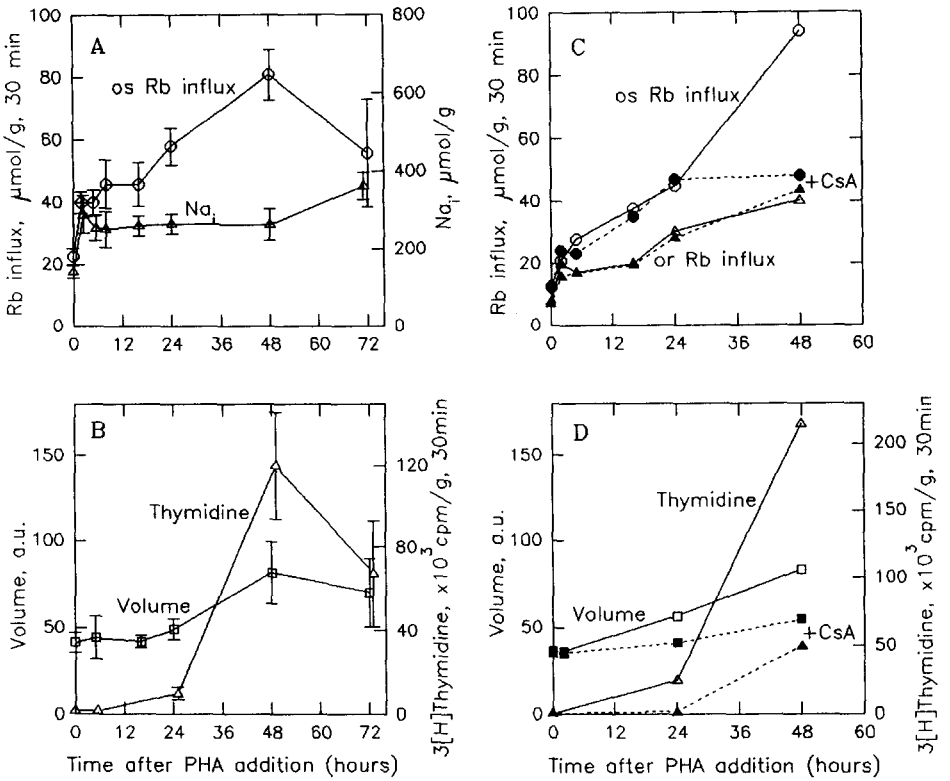


Fig. 1. Time course of ouabain-sensitive (os) and ouabain-resistant (or) Rb influxes, Na_i, DNA synthesis and cell volume in human lymphocytes activated by PHA alone (A,B) or by PHA with cyclosporin A (C,D). Open symbols, without cyclosporin A (CsA); closed symbols, with CsA (1 μg/ml). (A,B) The data are the means of 18–24 experiments; vertical bars represent the 95% confidence interval. (C,D) One representative experiment from six; each data point is the mean for triplicate cultures of lymphocytes from one onor.

The short-term stimulation of ion pump in animal cells activated by diverse mitogenic stimuli is considered to be due to elevation of Na_i [2,6,10]. Fig. 1A shows that PHA induces a twofold increase in Na_i within the first 2 h, however, between 5 and 48 h Na_i does not change. An increase in Na_i is observed at 72 h, just at the time when the Rb influx decreases. Therefore, the long-term increase in the ouabain-sensitive Rb influx cannot result from the elevated Na_i. There should exist another mechanism of up-regulation of the Na,K-ATPase pump during the cell cycle progression, which is independent of the Na_i.

To determine if the increase in the Na,K-ATPase pump activity associated with blastogenesis is controlled by genomic pathway, the inhibitors of translation (cycloheximide) and transcription (actinomycin D and α-amanitin) were used. When inhibiting completely the PHA-induced acceleration of protein synthesis, cycloheximide (CHX, 5 μg/ml) does not affect the early rise in ouabain-sensitive Rb influx but abolishes the influx increase after the 5th h of mitogenic stimulation (Fig. 2A and B). Addition of CHX to PHA-activated cultures during the progression stage (at 20 h) prevents the subsequent elevation of Rb influx (Fig. 2A). These findings indicate that as early as after 2 h of PHA stimulation and then in the course of lymphoblastogenesis the enhanced pump is dependent on a new protein synthesis.

When lymphocytes are stimulated by PHA in the presence of actinomycin D (0.004–0.04 μg/ml) or α-amanitin (4 μg/ml), the increase in ouabain-sensitive Rb influx remains unaffected as long as during 24 h of stimulation (Fig. 2C). However, at the second day the flux elevation becomes slower or it is completely suppressed, the degree of the flux inhibition being correlated with the degree of inhibition of total protein synthesis (Fig. 2C and D). The effect of the transcription inhibitors appears to be dependent on the stage of lymphocyte activation. Indeed, when added at the 20th hour of PHA stimulation, the drugs promptly abolish both the acceleration of RNA and protein synthesis and the late increase in Rb influx (Fig. 2C and D).

The time dependence of the inhibitory effect of actinomycin D and α-amanitin on PHA-induced pump stimulation seems to be consistent with the peculiarities of activation process in quiescent cells: in human lymphocytes like in other dormant cells at the initial stages of mitogenic activation protein synthesis is mostly controlled at the translational level [9]. It might be suggested that during the first day of mitogenic activation the newly synthesized pump proteins which are responsible for an increase in ion fluxes are translated from the preexisting Na,K-ATPase mRNAs, whereas at later cell cycle progression the generation of new pumps is also regulated at the transcriptional level.

Until recently, the rise in Na_i and the recruitment of plasma membrane pumps from an internal inactive pool have been proposed to explain an increase in the Na,K-ATPase pump activity in mitogen-stimulated lymphocytes [10,11]. These mechanisms are protein-synthesis independent and are operating during the early stage of lymphocyte activation. Taken together, our data demonstrate for the first time that (i) a new protein synthesis is required to provide for the enhanced transport activity of Na,K-ATPase pump during G0/G1/S transition; (ii) at a late stage of lymphocyte blastogenesis the protein

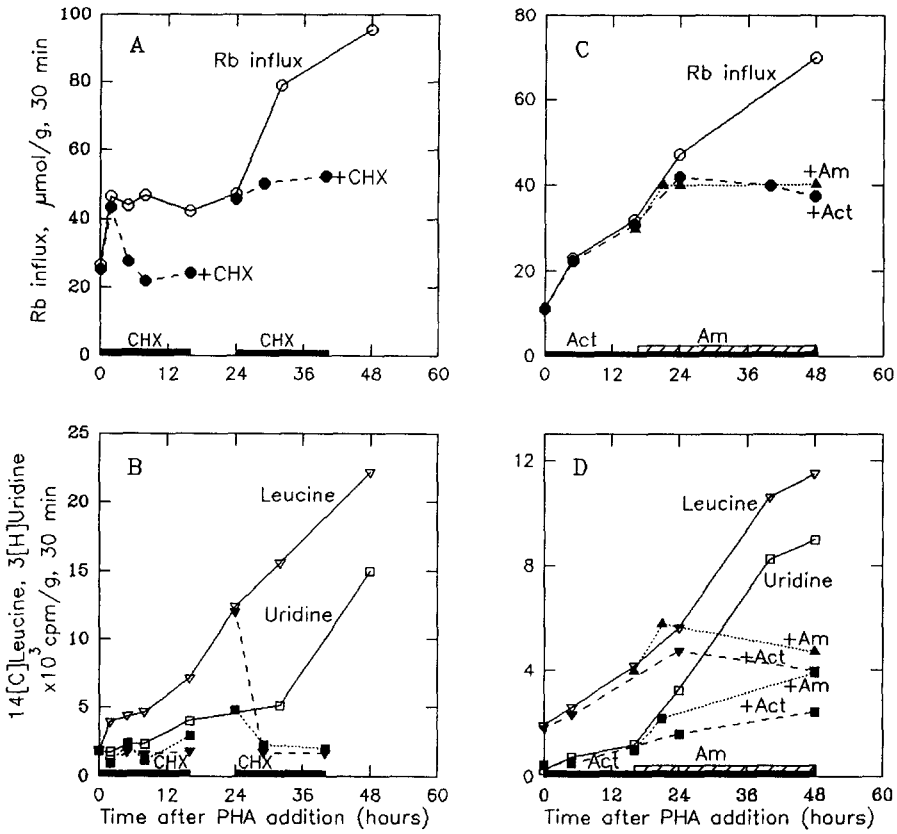


Fig. 2. Time course of ouabain-sensitive Rb influx under the inhibition of protein and RNA synthesis. (A,B) Effect of cycloheximide (CHX, 5 μg/ml). Solid lines and open symbols, no drug; dashed lines and closed symbols, in the presence of drug. To diminish the indirect effect of CHX on cation fluxes, it was administered during the periods not longer than 16 h (0–16 h or 24–40 h). (C,D) Effect of actinomycin D (Act D) and α-amanitin (Am). The same designations as in A,B. Act D (0.004 μg/ml) was added simultaneously with PHA at 0 time. Am (4 μg/ml) was added to PHA-activated cultures at 16 h. One representative experiment from eight. Each data point is the mean for triplicate cultures of lymphocytes from one donor.

synthesis-dependent increase in the pump activity is blocked under the inhibition of transcription.

At present, a few direct evidence is available as to the expression of Na,K-ATPase mRNAs in proliferating cells. Proliferation-related elevation of α1 and β1 subunit mRNAs has been reported for a serum-depleted-restored liver cell line and for the regenerating rat liver [12,13]. It has recently been found that the induction of mitogenic response in human lymphocytes is accompanied by an elevated level of Na,K-ATPase mRNAs at 8 h of PHA stimulation, i.e. well ahead of the growth-related activation of Na,K-ATPase pump [14]. Such an early expression of Na,K-ATPase mRNAs might underlie the activation of cation pump associated with the cell cycle progression.

In conclusion, previous studies of monovalent cation fluxes in mitogen-stimulated cells gave evidence for the notion that an early mitogen-induced activation of Na,K-ATPase pump was not obligatory for the initiation of cell proliferation [2,6]. Our current study demonstrates that the long-term up-regulation of Na,K-ATPase pump, concomitant to the growth phase of mitogenic reaction, is controlled at translational and transcriptional levels and might be considered as an essential event of cell progression from quiescence to proliferation.

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