Effect of Air Ions on the Membrane Na, K-ATPase Activity of L 1210 Cells

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Abstract. The effect of exposure of L 1210 mouse leukemia cells to artificially generated air ions on the activity of membrane Na, K-ATPase in the cells was investigated. The exposure of cells to air ions of both signs gave identical results, i.e. diminution of transport activity of the enzyme, measured by radioactive ^{86}Rb transport into the cell (ouabain-dependent). Passive ouabain-independent transport of Rb^+ into the cells remained unchanged in the air ion-treated cells, as did the passive efflux of the radioisotope from preloaded cells. The possible explanation of the phenomena observed is discussed.

Key words: Air ions — L 1210 — Membrane ATP-ase activity

Introduction

As shown in another paper, ouabain acts upon L 1210 mouse leukemia cells by diminishing the fluorescence of 1,8 aniline-naphthalene-sulfonate (ANS) in the membranes of these cells (Jaśkowski et al.). The effect was reversible when glycoside treated cells were subsequently exposed to an artificially generated "cloud" of negative air ions. Ouabain is known as a potent and specific inhibitor of the membrane Na, K-ATPase-"sodium pump" EC 3.6.1.3 (Moczydlowski and Fortes 1980; Smith and Vernon 1979; Kyte 1981; Hoffman et al. 1981). This enzyme actively transports sodium and potassium cations across the membrane (Kyte 1981; Hoffman et al. 1981). In some animal cells the activity of Na, K-ATPase participates in the generation of the electrical resting potential of their membranes and its restitution after the occurrence of the action potential (Kyte 1981; Freedman and Laris 1981). Changes in the composition of the ionic environment and concentration were suggested to influence the activity of the "sodium pump" (Kyte 1981; Ahrens 1981) but so far the possibility of a regulatory action of air ions on this activity has not been considered.

Materials and Methods

Mouse leukemia L 1210 cells growing intraperitoneally in syngenic DBA 2 mice were used. Washed cells were resuspended in an isotonic medium, containing 5.5 mmol . l^{-1} glucose, 137 mmol . l^{-1} NaCl, 1 mmol . l^{-1} $MgCl_2$ and 0.2 mmol . l^{-1} RbCl in redistilled and deionized water (Ledbetter and Lubin 1980; Bakker-Grunwald et al. 1980), at a concentration of 3.5×10^7 cells per ml. Six aliquots of cell suspension (0.1 ml) were placed in the wells of plastic macrophage migration plates (Sterilin UK). Three separate plates were prepared for each experiment: a reference plate, a negative ion-treated plate, and positive ion- treated one. Ouabain (Fluka, Switzerland) dissolved in the above medium was added to a final concentration of 10^{-4} mol . l^{-1} to three wells on each plate. The sample volume was adjusted to 0.2 ml with the same medium. The final thickness and the area of cell suspension in each well were 1 mm and 2 cm^2, respectively. The plates were placed in a shaked (100 rpm, 37 °C) water bath.

A BION −80 apparatus (Medicor, Hungary) was used as a stable source of negative and positive ions (5×10^{11} and 2×10^{10} ions per cm^3/per second, respectively) (Jaśkowski 1984). The distance between the edges of the ion generator and the surface of the cell suspension was 2 cm. The cells were exposed to air ions of a given sign for 45 min. All experiments were carried out in quadruplicate. The results were statistically treated with Student's *t*-test.

The determination of cation transport activity was done in a potassium free medium as described above, with the use of Rb 86 as a tracer of monovalent cation movement into the cell (Ledbetter and Lubin 1980; Bakker-Grunwald et al. 1980). About 0.9 MBq of ^{86}RbCl (Institute of Nuclear Research, Swierk, Poland, specific activity 128 MBq per ml) was added to each well containing cell suspension just before exposing the cells to air ions. The exposed cell suspensions were left to rest for another 15 min and subsequently transfered to separate plastic RIA counting vials. Cells were then washed 3 times with an ice-cold incubation medium (total volume 10 ml) and finally resuspended in 0.5 ml of the same medium containing 0.1 % Triton X-100. The incorporated radioactivity was counted in a LKB "Ultragamma" scintillation counter. The results were expressed as cpm or as percentage of control (reference) values.

Air ion-dependent efflux of monovalent cations from L 1210 cells was determined by measuring radioactivity stored in ^{86}Rb-preloaded cells after air ion exposure. A total of 3.5×10^7 cells were incubated in 1 ml of potassium free medium as above with about 2 MBq of ^{86}RbCl for 60 min in a 37 °C water bath. Labelled cells were washed 3 times with cold medium (total volume 10 ml) and resuspended in the same medium at a concentration of 3.5×10^7 cells per ml. As described above, 0.25 ml aliquots of labelled cell suspension were placed into plates and exposed to negative or positive air ions for 15 min (in some cases for 30 min): A control plate was run parallelly. The cells were then washed twice with cold medium and residual radioactivity was counted. The results of this part of the experiment were expressed as percentual difference between ^{86}Rb radioactivity remaining in untreated cells and that remaining in air ion treated cells.

Results

As shown in Fig. 1, the total transport of ^{86}Rb cations across the membranes of L 1210 cells diminished, when air ions of either sign were applied. The drop after the exposure to negative ions was highly significant ($p < 0.001$) while changes resulting from the application of positive ions were not ($p < 0.01$). As the passive Na, K-ATPase independent cation transport was not changed in air ion-treated

cells ($p<0.7$ for negative ions and $p<0.6$ for positive ions) the observed effect of air ions on total transport seems to be due to a drop in active Na, K-ATPase dependent transport. As can be clearly seen in Fig. 1, the value of active Rb^+ transport in L 1210 cells exposed to negative air ions for 15 min was significantly lower then that in control cells ($p<0.01$). Although the effect of positive air ions on the active transport of rubidium cations in the cells examined was similar it was insignificant ($p<0.3$).

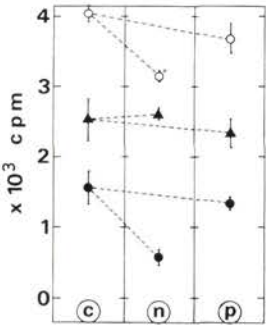

Fig. 1. The influence of negative and positive air ion application on $^{86}Rb^+$ transport through the membranes of L 1210 cells. Ordinate: radioactivity of ^{86}Rb incorporated into the cells; c — control, n — negative and p — positive air ion-treated plates. Open circles — total transport, black triangles — passive ouabain-independent transport and black circles — active ouabain-dependent transport.

The observed effect could, at least partially, be connected with changes in the passive flow of Rb^+ through the L 1210 cell membrane due to air ion action. The ability of air ion treated cells to store Rb cations was therefore checked. The cells were preloaded with ^{86}Rb and the radioactivity taken up by the cells was measured. As shown in Table 1, the effect of air ions was again unidirectional: both negative and positive ions caused an increase in Rb^+ content inside the treated cells by 3 and 8 %, respectively. These results were not significant but values obtained after 30 min of air ion exposure were markedly higher (Table 1).

Table 1. The influence of air ions on ^{86}Rb content of preloaded L 1210 cells. Each value represents the difference in terms of percentual value of control cells.

Duration of air exposure ion	NEGATIVE air ions	POSITIVE air ions
15 min	+3 %	+8.6 %
30 min	+22.1 %	+33.5 %

Discussion

The results of the present paper suggest the susceptibility of native Na, K-ATPase of mouse leukemia L 1210 cells to air ion action, resulting in changes of cation-transporting activity of the enzyme. Air ions of both signs caused a diminution of this activity, the effect of negative ions being much stronger than that of the positive ones. "Environmental" ionic changes have been suggested to play a role in the activity of membrane Na, K-ATPase (Kyte 1981; Ahrens 1981; Brotherus et al. 1980). The majority of data concerning this problem have been derived from changes in the lipid bilayer components surrounding (and functionally connected with) ATPase particles, with regard to the charge-bearing head groups of these lipid (Brotherus et al. 1980). ATPase depletion in the surrounding lipids in experiments with an isolated enzyme always resulted in inactivation of the sodium pump (Kyte 1981; Ahrens 1981; Brotherus et al. 1980). It is stressed that this inactivation is due not only to the lack of contact with lipid (s), but also to a drop in the negative charge surrounding the enzyme particles (Brotherus et al. 1980). In our work a similar effect was obtained by increasing the concentration of negative ions surrounding Na, K-ATPase. The transporting function of the sodium pump is regulated by changes in ionized sodium and potassium concentrations inside and outside the cells, respectively (Smith and Robinson 1981). It thus seems likely that other, extrinsic ions could also act as regulators of the pump function.

It is difficult to explain why air ions of both signs act upon Na, K-ATPase in the same manner. Perhaps, the transport activity of the membrane Na, K-ATPase remains in a state of a labile balance, which can be destroyed by even a slight change in the surrounding charge, irrespective of its direction. The possible biological significance of the phenomenon is to produce the action potential of the membrane in answer to differentiated stimuli. The development of action potential which is commonly observed in both nerve and muscle cells, has recently been described in mouse lymphatic cells by Kiefer et al. (1980) and the reaction of L 1210 cells (which are transformed mouse lymphoid cells) to exogenic stimuli might be of the same kind. For the generation of action potential, the activity of the membrane sodium pump must be stopped or at least diminished at the very first moment of the cell response (Smith and Vernon 1979). In the light of our experiments, the characteristic level of Na, K-ATPase activity in resting cells seems to be directly dependent on the specific values of surface charge and changes in the latter may produce a decrease in the enzyme activity.

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