Effect of Air Ions on L 1210 Cells: Changes in Fluorescence of Membrane-Bound 1,8-Aniline-Naphthalene-Sulfonate (ANS) after in Vitro Exposure of Cells to Air Ions

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Abstract. The ability of air ions to produce changes in the electrical properties of L 1210 mouse leukemia cells was tested. The fluorescence of ANS incorporated into a membrane lipid bilayer (measured microphotometrically) was used as a probe. It was shown that the action of air ions of both signs could change (negative ions by increasing and the positive ones by decreasing) the fluorescence intensity of ANS in the cell surface structures or to an imbalance of ions inside and outside the cell. Both possibilities are discussed in the light of the results of experiments using ouabain or biguanide as factors diminishing the intensity of ANS fluorescence.

Key words: Air ions — Tumor L 1210 — ANS fluorescence

Introduction

There is growing evidence that electrical charge of the cell surface (CSC) may play an important part in cellular physiology and pathology. We observed growth acceleration of B 16 melanotic melanoma induced by negative ions and an inhibiting effect when using positive air ions (Jaśkowski and Zbytniewski 1983). In addition acceleration effect of negative ions during the process of wound healing was also observed (Jaśkowski 1984; Jaśkowski and Myśliwski 1985). These results seem to suggest the possibility of a direct action of air ions in situ. Such an action should be reflected, first of all in changes in CSC, other physiological (clinical) effects observed after the application of air ions would be of secondary origin. Consequently, such changes should be detectable, and the aim of the present work was to try to detect them. The hypothesis of direct action of air ions on the cells has never been checked, probably due to some difficulties connected with the direct measurement of CSC, which had not been possible before the cell electrophoresis method was introduced (Pretlow and Pretlow 1979). Also, another electrical

parameter of the cell membrane, i.e. the transmembrane potential (tmp) was difficult to record using glass microelectrodes impaled individual cells (From and Schultz 1981). During the last few years, more simple techniques of CSC and tmp determination, or at least of recording CSC or tmp changes, have been developed. These methods use the electrophoresis apparatus for the determination of CSC changes exclusively (Pretlow and Pretlow 1979), and stable, strongly charged fluorescent dyes bound to (or incorporated into) cell membrane proportionally to the CSC or tmp, and slightly penetrating the cell interior (Shapiro et al. 1979; Grinvald et al. 1982). One of these dyes in ANS used with certain limitations for the determination of electrical surface charge of living cells (Schafer and Rowohl-Quisthoudt 1976; Njus et al. 1977). The present paper also deals with the problem of the use of ANS as a detector of CSC changes secondary to air ion application in vitro.

Materials and Methods

Mouse leukemia L 1210 cells growing intraperitoneally in syngeneic DBA/2 mice were used throughout the experiment. The cells were taken with the ascites fluid from the peritoneal cavity of ether-treated mice, washed three times with phosphate-buffered saline (PBS) and finally resuspended in this medium in concentration of 4×10^6 cells per ml. Aliquots (0.2 ml) of this suspension were transfered into wells of a plastic macrophage migration plate (Sterilin, UK) in shaken (100 rpm), 310 K water bath. The thickness of the cell suspension layers in the wells was 1 mm and the area about 2 cm^2.

As source of air ions of both signs, an apparatus BION-80 (Medicor, Hungary) was used. It had previously been tested by electrometric measuring of ion generation; it emitted 5×10^{11} negative ions and 2×10^{10} positive ions per 1 cm^3. The edges of the generator were placed 2 cm above the surface of the cell suspension. The cells were exposed to air ions of a chosen sign for 10—50 minutes (and, in some experiments, for maximum 90 min).

Ten min before the end of the exposure of cells to air ions, ANS in PBS was added to each well to a final concentration of 10^{-5} mol . l^{-1} ANS. Immediately after the end of ion application, a droplet of cell suspension was taken from the well and put onto a microscopic slide. Microphotometric measurements were done using a Reichert (Austria) microphotometer connected with a fluorescent microscope Zetopan of the same production, equipped with a Binolux II lamp. A Phillips Cs 200 W-4 high-preassure mercury lamp was used as the source of UV light; excitation wave length was 366 nm, that of emitted and measured light 486 nm (Jasaitis et al. 1971; Frehland 1982). The weak signal from the microphotometric unit was recorded by a digital millivoltmeter, and results were expressed as arbitrary units (AU) of incorporation.

As modulators of ion transport across the cell membrane, either 10^{-4} mol . l^{-1} ouabain (Fluka, Switzerland) or 2 mmol . l^{-1} 2-phenylethylbiguanidine (prepared as in Lodzińska et al. 1975) were used. One of these substances was added in appropriate concentration to each well just starting the exposure to air ions.

Results

As it was shown in Fig. 1, the exposure of living L 1210 cells to negative air ions resulted in a significant increment in the intensity of ANS fluorescence in the cell

membranes as compared to the control. The effect was visible and measurable from the very begining of the experiment, and the highest values of the ANS fluorescence were obtained after 25 min of exposure. Prolonged exposure of L 1210 cells to negatively charged air ions was followed by: (I) diminution of L 1210 membrane bound ANS fluorescence, (II) appearence of fluorescence in the cell interior (cytoplasm and organells), and (III) after 40—50 minutes of exposure to negative ions, cells started to grow larger and finally disrupted. The viability of cells during the first 25—35 minutes of exposure to negative ions remained unchanged (Trypan blue exclusion test).

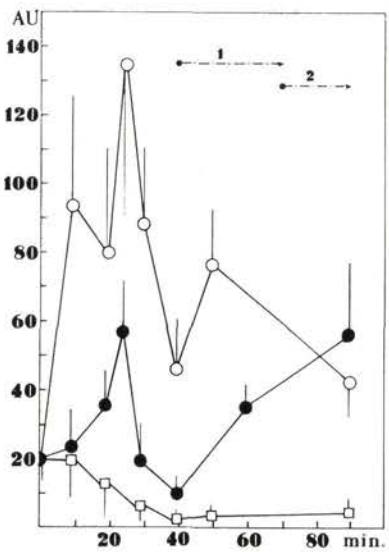

Fig. 1. The influence of negative or positive air ions upon the intensity of ANS fluorescence in L 1210 mouse leukemia cell membranes. Ordinate: intensity of fluorescence (arbitrary units, AU), abscissa: length of exposure to air ions (in minutes). Arrows → indicate: 1-start of ANS incorporation into the cytoplasm and organells, 2-start of cell disruption. ● reference (control) cell suspension ○ cells exposed to negative ion "cloud", □ cells exposed to positive air ions.

The effect of positive ions was of the opposite kind. As it was shown, the exposure of cell suspension to air ions of positive sign resulted in a significant decrease of fluorescence values, starting after 20 minutes of exposure. The diminution of ANS fluorescence measured in the membranes of positive ion-treated cells could be reversed (at least partially) by subsequent exposure of treated cells to negative ions.

The treatment with modulators resulted in both cases (Fig. 2) in a marked diminution of ANS fluorescence. Exposure of modulator (ouabain or biguanide) — treated cells to negative ion "cloud" resulted, in both cases, in the reversal of the drug action. This reversal was much stronger after ouabain.

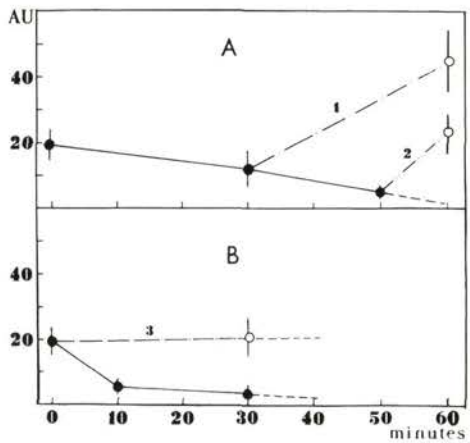

Fig. 2. Effect of 10^{-4} mol ouabain (*A*), and 2 mmol . l^{-1} biguanide (*B*) on the ANS fluorescence intensity in the membranes of L 1210 cells. Ordinate: fluorescence intensity (AU), abscissa: length of drug action (min). Note the reversal of drug effect following the exposure of cell suspension to negative air ions. ● drug alone, ○ -negative air ions applied starting at 30 minutes of contact with ouabain (1), after 50 minutes of contact with glycoside (2) and from the begining of the experiment with biguanide (3).

Discussion

The results presented above undoubtely show that the first reaction of living cells to a "cloud" of ions is a change in the fluorescence intensity of ANS incorporated in their membranes. ANS was used in this work as an indicator of changes in the cell surface charge (Schafer—Rowohl—Quisthoudt 1976). The rational underlying our interpretation of the results was as follows: (I) Acording to the schedule, the only parameter that changed was quantity of ions outside the cells regulated by the duration of exposure to generated air ions; (II) the intensity of ANS fluorescence before the damaging effect of negative ions occurred i.e. it increased proportionally to the time of exposure length during the first 30 minutes, it was thus proportional to the density of ions surrounding the cell. It reflected to a much higher degree, changes observed in the same time in control cell (see Fig. 1) suggesting paradoxically hyperpolarisation of the cell surface as a first reaction of the cell to changed external conditions. It fact, the oscilations of the cell surface charge during a reaction to external stimuli were observed (Uzgiris et al. 1983). It may thus be assumed that the changes in density of surrounding ions directly induced measurable changes in the electric properties of the cell membrane.

The nature of these changes remains obscure: they may be associated with simple adsorption of ions on the cell surface (and a direct change in CSC as a result), or they may be due to the disturbed balance between ion contents outside and inside the cell respectially i.e. to the generation or an additional transmembrane potential. The latter possibility seems to be likely in the light of data showing only a negligible sensitivity of ANS fluorescence to changes in membrane potential (Schafer 1978). However, the effect of both drugs used in the experiment, with ouabain bloking membrane Na, K-ATPase (Moczydłowski and Fortes 1980; Smith and Vernon 1979) and acting directly upon the membrane potential (Smith 1979),

and biguanide being a strong cation (Wróbel et al. 1979) which simply blocks negatively charged groups on the cell surface, indicates the complexity of the process.

Leaving this problem open until more data will be available it may be concluded that, in fact, air ions applied to the living cells in vitro, produce, first of all, measurable changes in the electric properties of their membranes which, in turn, might be the cause of physiological effects of air ions, observed in vivo.

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