CELL TO CELL POTASSIUM TRANSPORT IN A MONOLAYER OF MALIGNANT AND NORMAL RAT THYMUS CELLS

Eldad GIBERMAN, Avi KEYSARY and Shmuel GOTHILF
Istael Institute for Biological Research, Ness-Ziona, Israel

Received 4 October 1973

1. Introduction

In the formation of a multicellular organism a key process is the aggregation of individual autonomous cells to form colonies. Following aggregation, the cells in the organ differ from the original autonomous cells [1, 2]. This difference results from the mutual interactions of cells which come into contact with each other during aggregation. It seems very likely that these interactions depend primarily upon the cell membrane.

From studies of contact inhibition [2] and membrane conductivity measurements of cells in contact [1], there is a good deal of evidence to suggest that cancer and normal cells interact differently in a monolayer.

We decided therefore in the present work to study the transport of potassium ions from cell to cell through the regions of membranous contact of adjacent cells, in monolayer cultures of normal and malignant rat thymus cells.

2. Materials and methods

2.1. *Cells*

Both lines of cells were derived from the same pool of a rat thymus [3].

T21C, the control cell line, was grown in Eagle's minimal essential medium (Gibco F-11) supplemented with 10% fetal calf serum and buffered with bicarbonate to pH = 8.1. T21M, a cell line chronically infected with Moloney Leukemia Virus (MLV), was grown in the same medium and buffered to pH = 8.3.

A monolayer of cells was grown [3] on each of several microscope slides which were previously cut in halves. The slides were put inside a glass Petri dish [9 cm diam.] to which 20 ml medium and about 5×10^6 cells were added, and incubated in a 37°C humidified CO_2 incubator for 26 hr before the experiment.

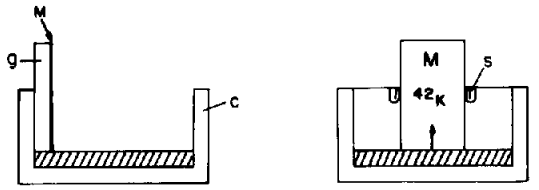

Fig. 1. A device for measurement of ion transport from cell to cell in a monolayer. Fig. 1a, side view; 1b, front view; c = stainless steel bath.(▨) filter paper (4×4 cm^2) wetted with a mixture of growth medium and ^{42}K solution. g = microscope glass slide. M = a monolayer of cells. S = spring to hold the slide vertically and in contact with the filter paper. (↑) Net propagation of $^{42}K^+$ ions through the cells. The net flow is in a direction perpendicular to the filter paper. The cells in the monolayer are exposed to the humidified atmosphere in the incubator during the experiment.

2.2. *Radioisotope*

^{42}K aqueous solution (contained 10 mM KCl, 140 mM NaCl, pH 7.3 and 400 μCi per ml) was obtained from the Israel Atomic Energy Commission. The γ-radioactivity was measured in a Packard Auto Gamma spectrometer.

2.3. *Directional cell to cell potassium transport*

Each slide was removed from the Petri dish and placed vertically, for different time intervals, in a stainless steel

North-Holland Publishing Company – Amsterdam

bath (fig. 1), on a sheet of filter paper which was previously wetted with 0.4 ml mixture of the growth medium, withdrawn from the Petri dish, and ^{42}K solution (10:1, v/v). The bath was then kept in a 37°C highly humidified (>95% relative humidity) CO_2 incubator, during the experiment.

At different time intervals the slides were removed from the bath, and washed twice in 6 cm diam. Falcon dishes with 6 ml of cold phosphate buffer saline (PBS) consisting of 3 mM KCl, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM sodium phosphate buffer at pH 7.3. The monolayer cells were then dissolved with 4 ml of 0.5 N NaOH.

The kinetics of the cell to cell ^{42}K isotope transport, in the upward direction (fig. 1b) was obtained from radioactivity measurements of 1 ml samples of dissolved cells which corresponded to the different slides.

Microscopic observations of the cells in the monolayers were performed before the slides were placed in the bath, as well as after they were washed with the cold PBS at the end of the experiment. No morphological changes and discontinuity in the monolayers, were observed. The monolayers were found to be uniform all over the experimental surface areas of the slides, in all cases. No bare area was found at the periphery of the surfaces boundaries. In parallel, cells were tested with trypan blue, after being exposed to the humidified atmosphere for 45 min. Cell damage was not detected during this period.

2.4. *Uniform potassium influx*

Monolayers of cells were grown on the glass slides, as described previously.

At the beginning of the K^+ influx experiment, 100 μl aliquots of ^{42}K solution were added to the growth media of the control and malignant cultures. At different time intervals, slides were removed from the growth medium and washed twice with 6 ml cold PBS. The cells were then dissolved with 4 ml 0.5 N NaOH, and the uptake kinetics of the ^{42}K isotope by the cells was obtained from radioactivity measurements of 1 ml samples of dissolved cells, which corresponded to different slides.

3. Results and discussion

The results of typical experiments are shown in figs. 2 and 3. The values given below are averages of three experiments.

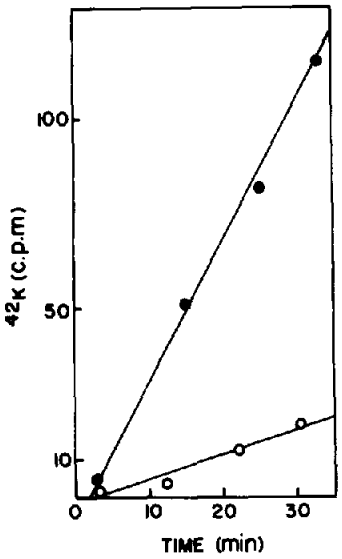

Fig. 2. Kinetics of ^{42}K transport from cell to cell in a monolayer of control and malignant rat thymus cells, at 37°C. (•) Control T21C cells. (○) malignant T21M cells. At zero time, the monolayer slides were placed vertically on a wet sheet of filter paper, in the bath (see text and fig. 1).

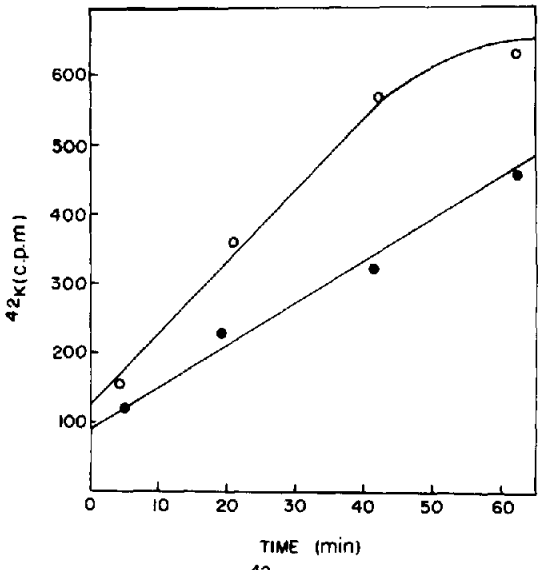

Fig. 3. Kinetics of uniform ^{42}K uptake by control and malignant rat thymus cells, in monolayer cultures, at 37°C. (•) control T21C cells. (○) malignant T21M cells. ^{42}K was added to the growth medium at zero time.

It was found that normal and malignant cells of rat thymus have different values of uniform K^+ influx. In the malignant cells the uniform influx was 80 ± 20% larger than in the normal cells (fig. 3).

However, as can be seen in fig. 2, the unidirectional cell to cell K^+ transport was at least 3-fold larger in the normal cells. In summarizing the present results, it should be noted that:

(1) The number of cells per unit area, in the monolayers, was found to be the same for malignant and normal cells.

Cytocrit values of cells which were previously suspended by trypsin from the monolayers, were within ±15%, the same for both types of cells. It was therefore reasonable to assume that the total surface area of the membranes as well as the average height of the cells above the glass planes in the monolayers, were the same for both types of cells.

(2) From the uniform influx experiments (fig. 3) it follows that isotopic ^{42}K equilibration [4], within the cells, has not been reached during a period of less than 1 hr, when the transport is through the membranes which face the growth medium.

(3) Considering the above ^{42}K isotopic equilibration time, the surface to volume ratio of the cells and the time interval of the directional cell to cell transport experiment (up to 30 min), it would seem reasonable to assume that only a few rows of cells which are closest to the ^{42}K paper, should be considered during the transport experiment.

(4) The K^+ transport being larger, and smaller, in the malignant cells than in the normal cells, in the experiments of fig. 3 and fig. 2 respectively, indicates that there was a junction between the membranes of neighboring cells. Otherwise, the K^+ influxes ratio of malignant and normal cells should have been larger or smaller than 1 in both experiments (figs. 2 and 3).

(5) Due to the background which resulted from the K^+ uptake by the cells in the first row, and which had (fig. 3) an opposite effect to that of the K^+ transfer from cell to cell, it was clear that the ratio of the uptake kinetics slopes (fig. 2) represent the lower limit for the difference in transport of K^+ ions from cell to cell, in the malignant and normal cells, in the present work.

It is of interest to compare our results with those obtained by Azarnia and Loewenstein [1] who measured the electrical resistance in a monolayer of cells, and found that the electrical conductivity from cell to cell is considerably larger in normal cells, than in cancerous cells.

Our results point in the same direction when the transfer of ions is considered between adjacent cells. However, the transfer of K^+ ions through the membranes which face the growth medium, was higher in the malignant than in the normal cells.

References

[1] Azarnia, R. and Loewenstein, W.R. (1971) J. Membrane Biol. 6, 368.
[2] Kohn, A. and Fuchs, P. (1970) Current Topics in Microbiol. and Immunol. 52, 94.
[3] Cremer, N.E., Taylor, D.O.N., Oshiro, L.S. and Teitz, Y. (1970) J. Nat. Cancer Inst. 45, 37.
[4] Giberman, E. (1968) Arch. Biochem. Biophys. 124, 543.