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CELLULAR ION CONTENT CHANGES DURING AND AFTER HYPERTHERMIA*

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SUMMARY: The potassium (K) level in mouse mastocytoma P815 cells undergoes a 40% reduction within 30 minutes of incubation at 43°C. It decreases further when the cells return to 37°C after a 60 minute 43°C incubation. A smaller change (20%) occurs after a 60 minute incubation at 41°C. Furthermore, nearly all of the lost K recovers in two hours after a subsequent incubation at 37°C. On the other hand, the sodium level in the cells increases by an amount much smaller than the potassium changes. However, the net loss of cations from the cells undergoing hyperthermia does not induce a simultaneous reduction of intracellular water volume.

Virus-transformed cells, compared to their normal counterparts, have been known to possess increased influxes of amino acids, nucleosides, sugars and potassium. It took Hatanaka's work (1) to show that the enhanced glucose uptake is not caused by the rapid intracellular turn-over rate of glucose, but by the increased permeability of the plasma membranes to glucose molecules. Likewise, elevated potassium transport and enhanced Na-K ATPase activity have been found for SV40 transformed 3T3 and BHK cells (2). Similarly, a two-to threefold increase in the rate of K efflux (3) as well as K influx (4) has been observed in human lymphocytes stimulated with PHA.

The enhanced membrane transports of particles by the growing cells must involve changes in the effectiveness and number of carrier molecules. The hyperthermia, on the other hand, increases the fluidity of the lipid phase and the flexibility of proteins. One may hypothesize that these two effects on the membrane are additive: the hyperthermia of growing cells induces flexibility of membrane molecules through which carrier molecules move more efficiently than at the normal temperature. One can further conjecture that the heat sensitivity of rapidly dividing cells originates, at least partially, from the additivity property of the above two effects.

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A possible consequence of an enhanced transport is a net transfer of molecules either out of or into the cells. If the flow of ions along their concentration gradient is sufficiently large to overcome the rates of their pumping against the gradient, then the sodium and potassium contents of cells must change. Indeed, the intracellular potassium was found to suffer a marked reduction at 43°C.

The present article is the first report of our investigations into the temperature effects on the membrane transports in tumor cells.

MATERIALS AND METHODS:

A. CELLS: Mouse mastocytoma cells transmitted in DBA/2 peritoneal cavity were removed by IP puncture and grown in spinner culture with spinner medium supplemented with 5-10% fetal calf serum. When the cell density reached $1 - 2 \times 10^6$ cells per ml of the culture medium, the cells were washed once and then resuspended in a fresh medium at a density of 10^6 cells/ml. Cell counts were made with a hemocytometer twice for each sample taken from the cell suspension before and after the incubation. Cell viability was tested by the dye exclusion method for all samples taken before and after the incubation.

B. HYPERTHERMAL INCUBATION: Cell suspensions divided equally into glass flasks were pre-incubated at 37.5°C for an hour before the flasks, except the controls, were transferred to a shaking incubator preheated at 41°C or at 43°C. Flasks containing culture media but with no cells in them were also incubated to be used as blanks for the later flame analysis. No measurable change of pH has been observed at either temperature if the cells were incubated in a 5% CO_2 atmosphere. The bath temperature was regulated to 0.1°C.

C. CELL WASHING AND LYSING: Ion permeation was stopped immediately after the incubations, or after a return to a two-hour 37.5°C incubation, by washing cells with choline chloride solution. Ice-cold isotonic choline chloride solution with 10^{-4}M oubain was added to the incubated cell suspension after the suspension was transferred to a precooled, 250 ml centrifuge tube for centrifugation at 2000-3000 g for 30 seconds (5). After aspirating out the supernatant, the cell pellet was resuspended in cold choline chloride solution to be washed again. After the hypotonic cell lysing, protein precipitation with TCA and a 20 minute centrifugation at 3000 g, the supernatant was then pipetted out of the centrifuge tubes. The pellet is washed and the washed liquid was combined with the previous supernatant. The liquid was evaporated completely and the residue was dissolved with concentrated HNO_3 and water added later. Then it was filtered through a 1.2μ filter placed over the sampling manifold (Millipore Corporation 1225). The filters were soaked in 10% HNO_3 and left in deionized water overnight before using.

D. FLAME EMISSION DETERMINATION OF IONS: Containers for all solutions used in the present flame emission analysis were rinsed with 10% nitric acid followed by a thorough rinsing with deionized distilled water. A Jarrell Ash Atomic Absorption Spectrometer (JA 82500) equipped with a new photomultiplier tube (Hamamatsu Type R 955) was employed in the emission mode with C_2H_2 as the fuel and air for the oxident. Both lysates and blanks were diluted by an equal factor so that the diluted lysates would have ion concentrations in the linear range of the % emission vs. concentration curve.

E. ATP ASSAY: Cells incubated at 43°C for 60 minutes were lysed and filtered. The filtrate, and the arsenate-phosphate buffer were mixed with the enzyme extract of firefly's tails. Immediately after the addition of the enzyme extract, the light pulses were counted with the liquid Scintillation Counter.
F. CELL VOLUME DETERMINATION: This procedure was adopted from DuPre and Hempling (6).

RESULTS

The ion contents of cells depend on many interrelated variables such as cell volume, culture temperature, cell density, freshness of the medium and type of serum. In a rapidly growing medium and at an optimum temperature, the potassium content of cells at a higher density was lower than that of cells at a lower density.

Neveretheless, the fractional decrease of the potassium content of growing cells stayed approximately the same independent of types of medium or serum. Thus, cells incubated at 43°C for 30 minutes have suffered a loss of 40% in their potassium content. Cells incubated at 41°C for 60 minutes have lost approximately 20% of their potassium.

Cells subjected to the 41°C hyperthermia for 60 minutes recovered most of the lost ion content after a two-hour period of recovery. In sharp contrast, the potassium lost after 60 minutes of incubation at 43°C did not recover after a two-hour incubation at the physiologic temperature. The potassium level of these cells decreased further as shown in Figure 1.

The sodium contents of cells that have undergone a hyperthermal incubation at either 43°C or 41°C were higher than the control group. However, the quantitative changes were less definitive than those of potassium. The net change of the sodium is very small compared to that of the intracellular potassium. No change in the dye-taking fraction has been observed during the incubation.

The intracellular ATP and water fraction did not change after the cells were incubated at 43°C for 30 or 60 minutes.

DISCUSSION

A large decrease in potassium and a small increase in sodium inside the cells must accompany an efflux of anions to preserve the charge neutrality of the individual cells. The net reductions of both intracellular cation and anion contents must also drive water out of the cells to prevent osmotic imbalance. The volume of the cell, in turn, ought to decrease. However, no evidence for the water movement or the cell volume change has been found.

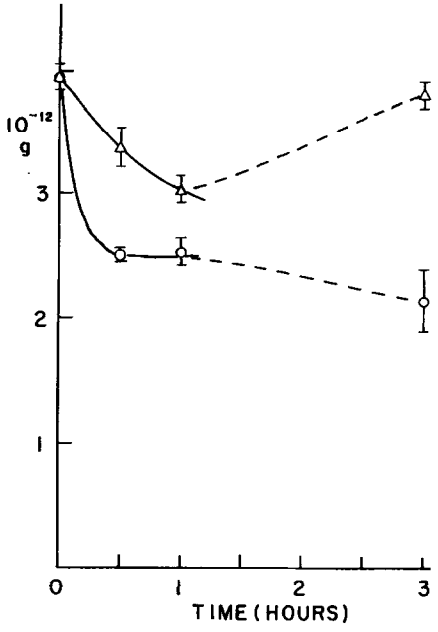

Figure 1. The amount of potassium per cell changes as a function of the incubation time at 41°C (Δ) and at 43°C (o). The lost potassium returns to cells which are subsequently incubated at 37°C after a one hour 41°C incubation. The potassium level continues to decline for cells which were incubated at 43°C for 1 hour.

In the above description water was assumed to follow the movement of potassium. On the other hand, one can also assume that water movement is the primary event, and that the potassium follows water merely to maintain the osmotic balance. This assumption is plausible because the lower entropy state of the cell interior water implies that the chemical potential of the cell water does not decrease as rapidly as that of the exterior water as the temperature is raised: i.e.,

$$\left(\frac{\partial S}{\partial n_w}\right)_T = -\left(\frac{\partial \mu_w}{\partial T}\right).$$

Therefore, water would flow out of the cells as the temperature of the cells is increased. The cell water masses measured after incubations at 37°C and at 43°C were, however, identical. Direct measurements of cell diameters using a microscope indicate no sign of their temperature dependence. It is, therefore, possible that the water that leaked out during a hyperthermal incubation returns into the cell interior during the water content and diameter determinations. Then, the potassium doesn't follow the water because the cells incubated at 43°C for 60 minutes lose more potassium during subsequent incubation at the normal temperature.

Assuming that the ion content changes may be interpreted as the concentration changes, one can distinguish two possible routes for the outcome. The first possibility is the simple diffusion of ions along the concentration gradient. Thus, the potassium ions flow from the cellular interior to the exterior and the sodium ions diffuse in the opposite direction. The second possibility is the lower activity of the ion pumps. Since the intracellular ATP level does not change at hyperthermal temperatures employed in the present experiments, one should look elsewhere to find the cause for the slowing of the ion pumping.

The unknown mechanism involved in this ion content change must explain the asymmetry between the potassium loss and the sodium gain. Only one sodium ion was gained for every 6 or more potassium ions lost. This occurs despite the concentration difference across the membrane being greater for sodium than for potassium. Of course, a small content change does not imply a small ion flux. It is quite possible that sodium ion fluxes into and out of the cells are both large, but nearly equal giving a small net change in the ion content of the cells. The ion flux measurements are in progress.

The ionic cellular response to the environmental temperature of 41 - 43^{o}C is not limited to tumor cells. The mouse spleen lymphocytes incubated similarly exhibit a loss of 60% of the original potassium and a gain of 20% of the original sodium content of the cells.

Such cellular responses are not universal. The human red blood cells incubated in whole blood or in a saline solution up to 44^{o}C do not exhibit any loss of potassium or any gain of sodium.

It is not clear at this time whether the ion movement across the plasma membrane during a hyperthermia is a symptom of a passive heat injury of cells or an active response of the cellular defense against a heat damage. To differentiate these two possibilities, incubated cells are treated with a potassium-rich saline solution and the survival rate is measured to compare with the survival rate of the cells treated with a regular saline. This investigation is in progress.

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