ENHANCEMENT OF POTASSIUM INFLUX, IN BABY HAMSTER KIDNEY CELLS AND CHICKEN ERYTHROCYTES, DURING ADSORPTION OF PARAINFLUENZA 1 (SENDAI) VIRUS

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1. Introduction

Adsorption of ultraviolet-inactivated Sendai virus causes a marked but transient inhibition of cellular deoxyribonucleic acid synthesis in HeLa [1], baby hamster kidney (BHK 21), FL, 1-929 and Chinese hamster cells [2].

Sendai virus adsorption also affects the chemical properties of cell membrane (Glick, Fuchs and Kohn, unpublished data).

In view of reports showing the inhibitory effect of high potassium concentration on cell division and deoxyribonucleic acid synthesis, in animal cells [3, 4], it was desirable to see whether virus adsorption had any effect on potassium influx in animal cells.

2. Materials and methods

2.1. *Cells*

BHK 21 cells (clone 13), were grown in a monolayer, in Eagle's minimal essential medium (MEM, Grand Island Biological Co. F-12) supplemented with 10% calf serum [5].

During the experiment, the cells were at the logarithmic phase of growth. $1-1.2 \times 10^6$ cells were seeded in each Falcon dish (60 mm diameter) with 4 ml growth medium, and grown to about 2×10^6 cells per plate, in a CO_2 incubator at 37°. Cells were counted in replicate plates, after trypsinization.

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2.2. *Red blood cells*

(C. RBC) were obtained from a chicken heart, 1 hr before the experiment. Coagulation was prevented by heparin. The cells were washed twice and resuspended to about 5% hematocrit in a mixture (1:3, v/v) of chicken plasma and 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.

2.3. *Virus*

Parainfluenza 1 (Sendai) virus, inactivated by U.V. irradiation was prepared as described previously [1].

For experiments with C. RBC, the viral hemolysin was inactivated by heat treatment (45° for 40 min).

2.4. *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.

2.5. *Virus adsorption and potassium influx*

2.5.1. BHK cells

After decantation, 640 hemagglutination units (HAU) of U.V. inactivated Sendai virus in 0.2 ml PBS were added to each of the culture dishes designated to interact with the viruses. Similarly, 0.2 ml PBS were added to the control, untreated cultures. All the cultures were then returned to the incubator. 15 min later, 2 ml of prewarmed MEM solution was added to each dish. At the beginning of the K^+ influx experiment, 15 or 45 min after the treatment with viruses

started, a 50 μl aliquot of ^{42}K solution was added to each culture. At different time intervals, culture dishes were washed twice with 5 ml cold PBS. The cells were then dissolved with 2 ml sodium dodecyl sulfate as described previously [1].

The uptake kinetics of the ^{42}K isotope by the cells was obtained from radioactivity measurements of 1 ml samples, of dissolved cells.

During the ^{42}K uptake experiment, the culture plates in the incubator were shaken gently from time to time. The radioactivity which remained in the extracellular liquid, after washing, was found to be negligible.

2.5.2. C. RBC

One ml aliquots of C. RBC suspensions, in 13 mm diameter tubes, to which either ouabain or 3200 HAU inactivated virus, or a mixture of 3200 HAU inactivated virus with ouabain, were added respectively, were left at 21° for 20 min. PBS was added to the control 1 ml C. RBC suspension. The final concentration of ouabain was 1 mM.

The tubes were then incubated at 37° with gentle shaking, and 50 μl aliquot of ^{42}K solution was added to each of them.

The uptake kinetics of the radioisotope by the cells was determined from radioactivity measurements of the RBC pellets, obtained from samples removed at different time intervals from the C. RBC suspensions. The cells were separated from the extracellular medium by the differential flotation method, as described previously [6]. Separations were carried out in 0.4 ml polyethylene tubes. Each tube contained 0.05 ml of the separating fluid (density of 1.066 g/cc) to which 0.1 ml of the RBC suspension was added, and centrifuged for 2 min at 10^4 *g* in a Beckmann 152 microfuge.

In a separate experiment, it was found that incubation with ouabain for 20 min did not effect C. RBC agglutination by Sendai virus. Hemolysis of C. RBC treated with viruses, was not detected during the experiment.

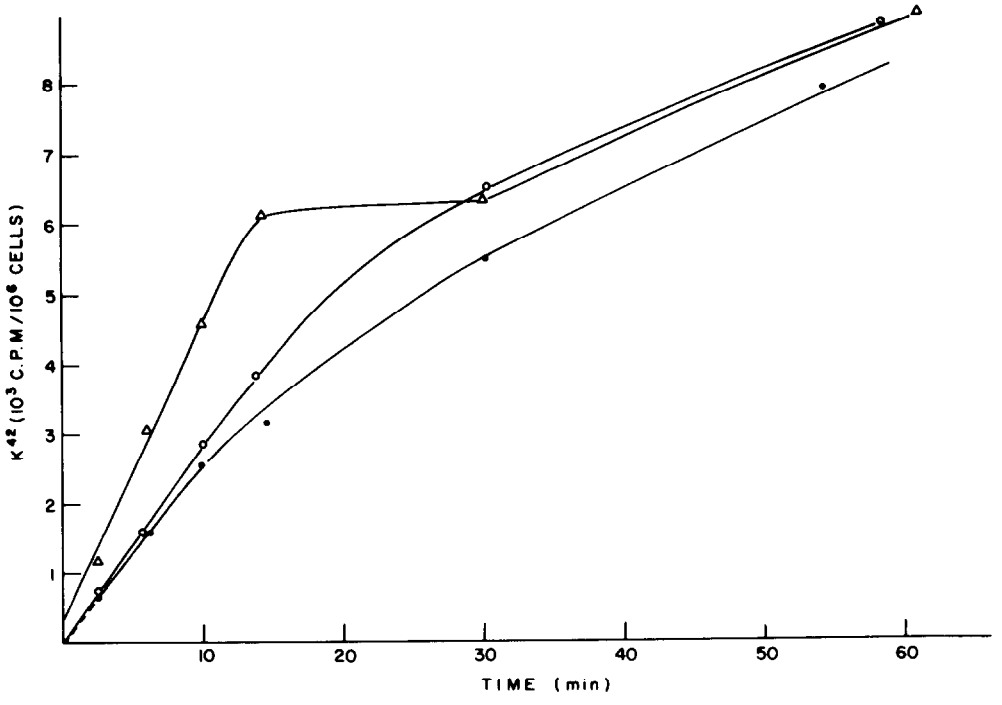

Fig. 1. Kinetics of ^{42}K uptake by treated and control BHK 21 cells, at 37°. (○—○—○) Control; (△—△—△) 15 min after treatment; (●—●—●) 45 min after treatment.

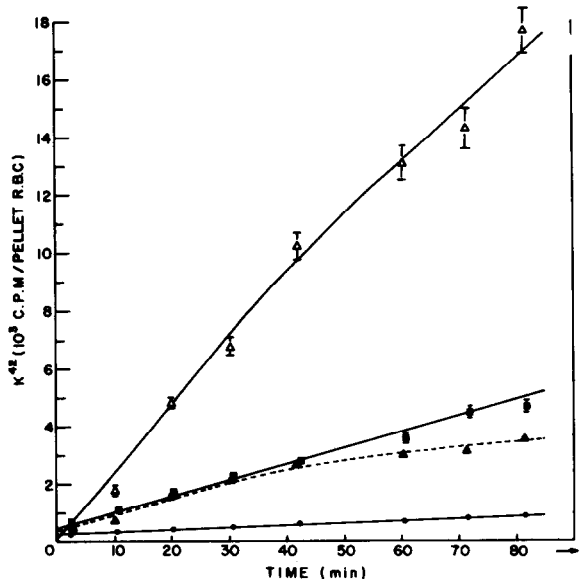

Fig. 2. Kinetics of ^{42}K uptake by treated and control C. RBC, at 37°. (■—■—■) Control; (●—●—●) control with ouabain; (△—△—△) 20 min after treatment; (▲—▲—▲) 20 min after treatment with ouabain. Ouabain concentration was 1 mM. After the time interval of virus interaction with the cells, ^{42}K was added to the suspensions (zero time). The viruses remain with the cells during the influx experiments.

3. Results

The results of typical experiments are shown in figs. 1 and 2.

Following the treatment of cells with Sendai viruses, enhancement (4.1-fold) of the total influx and active transport of K^+ in chicken RBC, was obtained, at 37°. K^+ influx inhibition due to ouabain was 87% and 85% in the cells which were treated and untreated, respectively. Successive periods of K^+ influx enhancement (1.6-fold), levelling off, and a recovery to the original value before the virus treatment, were observed in BHK 21 cells at 37°.

The decrease in the influx started at about 30 min after the beginning of the virus treatment, and lasted for 5 min.

K^+ influx has not recovered to the original value, in chicken RBC, during the experiment.

In BHK 21 cells, at 4°, K^+ influx was not affected by virus adsorption.

The estimated errors in the relative influx values were less than 10%.

4. Discussion

Although the nature of virus adsorption to membranes of animal cells is unknown in many cases, it was rather suggestive to expect leaks in the membranes, at some stages of the adsorption. It was therefore attractive to correlate the enhancement of K^+ influx in BHK cells, obtained in the present work, with leaks in the membranes.

The influx enhancement was followed by a period of negligible potassium uptake. There was therefore a damage to active transport of K^+ during that period, which later on recovered to the level of the normal untreated cells.

It is quite plausible that the K^+ active transport damage resulted at an earlier stage, and potassium uptake during the enhancement period was mainly due to leaks in the membranes. However, results obtained at 4° indicate that most of the influx during the enhancement period was an active one. In experiments with C. RBC, inhibition of the influx by ouabain served as criterion for active transport of potassium. K^+ influx, which increased 4-fold in treated RBC, was nevertheless inhibited with ouabain to the same degree as normal RBC.

K^+ influx has undergone about 85% inhibition in both treated and untreated C. RBC, when ouabain was added. The marked similarity of the inhibition values in normal and virus C. RBC, makes it unlikely that ouabain was just decreasing leaks of K^+, in some way, in the treated cells. Following this argument, it is proposed that ouabain inhibited active transport of K^+ in treated cells, and that active transport of K^+ was enhanced in C. RBC treated with inactivated Sendai virus.

A rise in K^+ active transport activity might have resulted from either conformational changes in the structure of the host membrane, or from increased number of sites for active transport, due to the fusion of the virus envelope with the membrane of the treated cells (see below) [7] assuming that the original virus envelope has active transport sites for K^+. The average number of viruses, the linear dimension of which is about 200 nm, adsorbed to a single C. RBC,

as calculated from its hemagglutinin titer, was 6 ± 3. The astonishing large enhancement of K^+ influx supports a hypothesis that the transport activity per surface area is considerably larger in virus envelopes than in host membranes.

It is quite possible also that during fusion, ATPase present in the viral envelope enriches the host membrane with energy available for active transport.

Adsorption of paramyxoviruses to a variety of cell lines cause a transient inhibition of cellular DNA [1] and protein synthesis (Grossman and Kohn, to be published). This inhibition lasts from about 30 to 90 min, post adsorption [1]. In BHK 21 cells, we therefore checked, in parallel with K^+ influx experiments, the ability of virus to induce inhibition of cellular DNA synthesis. In all the experiments reported above, a marked inhibition of 40 to 50% was observed.

Since DNA and protein inhibition were both early and transient, and were induced not only with whole inactivated viruses but also with purified hemagglutinins, and since concomitantly with the inhibition, a significant increase of K^+ influx also occurred, the following model may be suggested: A direct interaction occurs between the viral envelope and cell membrane. This interaction leads to fusion of the two and results in chemical and conformational changes in the cell membrane. These changes may serve as a signal affecting the regulation of the synthesis of macromolecules in the host cell [8].

Could a change of the ratio K^+ to Na^+ serve as such a signal? Cone [3] and Orr et al. [4] have reported that changes in this ratio inhibit cell multiplication and DNA synthesis. From considerations related to influx and time of cellular potassium exchange values, we estimate variation of potassium intracellular concentration, during viral treatment of BHK 21 cells, to be relatively small (< 50%), in the present work. It is our opinion therefore that regulation of macromolecules synthesis, in the BHK 21 cells, is not affected via potassium concentration change, in our experiments.

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