The Enhancing Effect of Copper, Nickel, and Cobalt Ions on Plaque Formation by Semliki Forest Virus (SFV) in Chicken Embryo Fibroblasts

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With 4 Figures

Received September 5, 1974

Summary

In cultured chicken embryo fibroblasts treated with copper-(copper cation $10^{-3.5}-10^{-5}$ M), nickel and cobalt salts an enhancement of the plating efficiency of Semliki Forest virus could be observed. This augmented plaque formation was most probably due to a higher adsorption rate of virions to the cell surface under the influence of the transition metal ions. The plating efficiency of West Nile virus in chicken-embryo fibroblasts and, to a lesser degree, of poliovirus type 1 and 2 in KB-cells was also enhanced by copper sulfate.

1. Introduction

In the course of stabilizing experiments with semi-purified interferon preparations, several metal salts were tested for toxicity in chicken embryo fibroblasts. These experiments revealed that a treatment of the cell layer with certain metal ions led to higher plating efficiency of Semliki Forest virus (SFV). There is strong evidence that the enhancing effect is caused by an increase in the adsorption rate of SFV-particles to the surface of cells.

2. Materials and Methods

2.1. Virus Strains


Poliovirus type 1, 2, 3 freshly isolated from clinical material, 2nd passage rhesus monkey kidney cells.

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2.2. Metal Salts

These were all analysis grade (Merck, Darmstadt) and Komplexon III (EDTA) (Siegfried, Zofingen) was used for complexing. As a diluent for metal salt solutions and for virus suspensions we used Hanks-Albumin containing 0.05 per cent bovine serum albumin (BSA, Armour, Eastbourne) (5).

2.3. Cell Cultures and Plaque Assay

The methods used for producing chicken fibroblasts were described previously (10, 11). Instead of NaHCO₃, Tris or HEPES buffers were used and cultures were not aerated with a CO₂-air mixture. KB-cells and monkey kidney cells were cultured in Eagle’s basal medium Hanks’ base (BBL, Cockeysville) and the plaque assay was performed as described by DARELL (4) and HOLLAND and MCLAREN (7).

2.4. Metabolic Inhibitors

For the inhibition of cell RNA and protein synthesis actinomycin D, cycloheximide, puromycin and DL-p-fluorophenylalanine (Calbiochem, Lucerne) were used.

2.5. Viral RNA

For the extraction of viral RNA from brains of infected baby mice and for infection of cell cultures with viral RNA, we followed the methods described by SANDERS (21) and CLICK and HACKETT (3).

2.6. Protein Estimation

Lowry’s method was used (15).

2.7. Statistical Evaluations

Calculations were determined according to LORENZ (14).

2.8. General Procedure in Conducting the Experiments

As a rule, the cell monolayer was overlayed with 2 ml of the metal salt solution for 3½ hours and the supernatant removed. Then 1 ml of a SFV-suspension with a definite number of PFU/ml was added and left in contact with the cells for 1½ hours at 35°C, following which the supernatant fluid was replaced with an agar overlay and the dishes were incubated for 48 hours at 35°C. Plaque readings after the addition of high multiplicities were made before plaques grew to confluency. Values given in the figures and tables are based on experiments performed in triplicate using 3 dishes per metal salt concentration.

3. Results

3.1. Influence of Different Metal Salts on Plaque Formation by SFV

The experiments were performed as described in section 2.8. The results are shown in Table 1. With copper, nickel and cobalt salts higher plaque counts were achieved. Plaque formation was best enhanced with copper salts (3—10 fold) but the concentration range was restricted (relative to cation 10⁻³.₅—10⁻⁵ M) (23). Morphology of the plaques seemed unchanged and there was no strict linear relationship between salt concentration and the number of plaques. The anions (Cl⁻, SO₄⁻⁻) had no influence on enhancement effect (20, 23). Nickel and cobalt ions were active only at near toxic concentrations, and after the application of these salts the size of the plaques was reduced.

With ZnCl₂ and AuHCl only marginal effects could be observed (enhancement 80 per cent). Ag⁺⁺, Cd⁺⁺, Fe⁺⁺, Mn⁺⁺, Mo⁺⁺, Ti⁺⁺, Ca⁺⁺, Mg⁺⁺ were not effective.