Tacaribe Virus Infection May Induce Inhibition of the Activity of the Host Cell Ca\(^{2+}\) and Na\(^{+}/K^{+}\) Pumps

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SUMMARY

Infection of Vero cells with Tacaribe virus stocks containing a high ratio of standard (plaque-forming) viruses to defective interfering particles (DIP) induced inhibition of the host cell Ca\(^{2+}\) ATPase (Ca\(^{2+}\) pump) and the ouabain-sensitive Na\(^{+}/K^{+}\) ATPase (Na\(^{+}/K^{+}\) pump). The Mg\(^{2+}\) ATPase which is not involved in cation transport was not affected. The presence of DIP in the inocula protected the cells from alteration of the transport-associated ATPases induced by standard viruses.

Virus infection of eukaryotic cells may result in numerous effects on plasma membrane functions such as inhibition of the transport systems for ions and small molecules, alterations in membrane potential or fluidity and breakdown of permeability barriers. These virus-mediated plasma membrane effects are likely to contribute to cell death in lytic infections, they may underlie perturbations of cellular functions, or promote the expression of malignant phenotypes by transformed cells (Pasternak & Micklem, 1981; Garry et al., 1982; Carrasco & Lacal, 1983; Lutton & Gauntt, 1986; Seth et al., 1987).

In this report we study the effect of infection by an arenavirus, Tacaribe virus, on the activity of two plasma membrane enzymes involved in the active transport of cations; the Ca\(^{2+}\) ATPase (Ca\(^{2+}\) pump) and the ouabain-sensitive Na\(^{+}/K^{+}\) ATPase (Na\(^{+}/K^{+}\) pump) (Jørgensen et al., 1982; Roufogalis et al., 1982).

The arenavirus group contains enveloped viruses with segmented ssRNA genomes which easily establish persistent infections (for a review see Howard, 1986). They may cause perturbations of specialized functions in differentiated cells, as demonstrated during in vivo and in vitro infections by lymphocytic choriomeningitis virus (LCM virus) (Oldstone et al., 1977, 1984a, b; Valsamakis et al., 1987). In addition, standard (plaque-forming) arenavirus particles may induce alterations of vital cellular functions and cell lysis in certain cell lines (Dutko & Pfau, 1978; López & Franze-Fernández, 1985).

To explore whether Tacaribe virus infections affect host cell Ca\(^{2+}\) and Na\(^{+}/K^{+}\) pumps, exponentially growing Vero cells were mock-infected or infected at an m.o.i. of 10 with Tacaribe virus stocks containing a ratio of p.f.u. per defective interfering particle (DIP) of greater than 100. The preparation of virus inocula and infection of the cells was performed as previously described (López & Franze-Fernández, 1985). At the indicated post-infection (p.i.) times, approximately 1 \times 10^6 cells from either mock-infected or infected dishes were washed three times with a cold solution of 150 mM-NaCl. The cells were detached by scraping in the presence of the same solution, transferred to a centrifuge tube and pelleted at 3000 g for 3 min. The cell pellet was resuspended in 0.15 ml of double-distilled water and homogenized in a Potter-Elvehjem microhomogenator. Triplicate samples of the crude homogenate preparation were used to measure the Ca\(^{2+}\) ATPase activity, the ouabain-sensitive Na\(^{+}/K^{+}\) ATPase activity and

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Fig. 1. Effect of Tacaribe virus infection on ATPase activities. ATPase assays were performed as previously described (Rossi et al., 1985) in 0.5 ml (final volume) of a medium containing 30 mM-Tris-HCl (pH 7.4 at 37 °C), 20 mM-KCl, 3.5 mM-MgCl₂, 130 mM-NaCl, 2 mM-[γ-³²P]ATP, together with the components indicated below for the assay of each ATPase activity. The media also contained 60 to 150 μg of protein per ml from the cell homogenate. For the Ca²⁺ ATPase activity assay (▲) the medium contained 0.1 mM-ouabain and enough CaCl₂ to obtain a final concentration of 40 μM-Ca²⁺. The Ca²⁺ ATPase activity is the difference between the activities in media with and without Ca²⁺. For the Na⁺/K⁺ ATPase assay (○) the reactions were performed in the absence or presence of 0.1 mM-ouabain in media without Ca²⁺ (1 mM-EGTA). The ouabain-sensitive ATPase activity represents the Na⁺/K⁺ pump. The Mg²⁺ ATPase activity (●) is the difference between the activity in a medium containing 0.1 mM-ouabain and 1 mM-EGTA, and that in the same medium without the addition of the protein homogenate. Protein concentration was determined by the method of Lowry et al. (1951). The ATPase activities were expressed in μmol Pi/mg protein since the protein content (0.6 mg protein is equivalent to 1 x 10⁶ cells) in mock-infected and infected cells showed no variations at least up to 28 h p.i. The results are the mean of three independent experiments. Vertical bars represent standard errors.

the Mg²⁺ ATPase activity. In these in vitro assays, possible changes in ATP, Pi or cation concentrations arising as a consequence of virus infection are not reflected in the activity of the enzymes. In addition, since the Mg²⁺ ATPase is a membrane protein which has been described as an ectoenzyme not involved in cation transport (Hamlyn & Senior, 1983), its activity constitutes a good internal control of the viral effect on transport ATPases. Two different characteristics of the Ca²⁺ ATPase activity measured in our assay medium indicated that this activity mainly belonged to the plasma membrane Ca²⁺ pump; firstly the Ca²⁺ ATPase activity was inhibited by up to 80% by compound 48/80 (Kᵢ = 9.9 ± 2.5 μg/ml); this drug has been described as the most specific and powerful inhibitor of the calmodulin effect in different eukaryotic cells (Gietzen et al., 1983; Gronda et al., 1987). Secondly the enzyme activity was inhibited to a high extent by vanadate, with a Kᵢ of 4.75 ± 0.75 μM, a value close to that found for the inhibition of the plasma membrane Ca²⁺ ATPase from human red cells (Rossi et al., 1981) and cardiac sarcolemma (Morcos, 1982). In addition, the Ca²⁺ ATPase was not affected by 20 mM-NaNO₃ which abolishes the mitochondrial ATPase activity (Formby et al., 1976). The results displayed in Fig. 1 show that virus infection induced a steady decline in host cell Ca²⁺ ATPase and Na⁺/K⁺ ATPase activities, but the Mg²⁺ ATPase activity was not significantly affected. Considering that the time required for the synthesis and release of an arenavirus particle is 6 to 8 h (Howard, 1986; López et al., 1986), the virus-mediated inhibition of the transport-associated ATPases represents an early event after infection. On the other hand, it is important to mention that the alteration in the Ca²⁺ and Na⁺/K⁺ pump activities took place well before the first signs of cytopathic effect which were evident at 30 h p.i. It should be pointed
Table 1. Effect of DIP on Tacaribe virus-induced inhibition of ATPase activities

<table>
<thead>
<tr>
<th>Cells*</th>
<th>ATPase activity† (µmol Pi/mg protein per h)</th>
<th>Virus yield‡ (log particles/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺/K⁺</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td>P.f.u./cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.01</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>3.0</td>
<td>0.40 ± 0.03</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>6.0</td>
<td>0.37 ± 0.03</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Mock-infected</td>
<td>0.46 ± 0.02</td>
<td>0.21 ± 0.01</td>
</tr>
</tbody>
</table>

* Exponentially growing Vero cells were mock-infected or infected as indicated (López & Franze-Fernández, 1985). The inocula were prepared as previously described (López & Franze-Fernández, 1985).
† ATPase activities were assayed as in Fig. 1 at 24 h p.i. Mean ± standard error of three determinations is shown.
‡ Assay of standard virus and DIP were performed as indicated (López & Franze-Fernández, 1985) at 24 h p.i. Mean value of three (p.f.u.) or two (DIP) independent titrations each performed in duplicate dishes. Range is shown in brackets.

out that the experiments were performed under conditions in which all cells in the culture were infected, as the results of immunofluorescence tests indicate (data not shown), and that infection with u.v.-inactivated viruses did not affect the ATPase activities (data not shown).

The role of DIP on Tacaribe virus-induced inhibition of the Ca²⁺ and Na⁺/K⁺ ATPase activities was also investigated (Table 1). Vero cells were co-infected with standard Tacaribe virus (10 p.f.u./cell) and with different amounts of DIP (0.01, three and six DIP/cell). At 24 h p.i., host cell Ca²⁺ ATPase, Na⁺/K⁺ ATPase and Mg²⁺ ATPase were assayed, and the intracellular plus extracellular virus particles (p.f.u. and DIP) were titrated. The results indicated that DIP protected Vero cells from the inhibition of the Ca²⁺ and Na⁺/K⁺ ATPase activities induced by standard viruses. It was also found that although both standard virus and DIP replicated in cells infected with the different inocula, the yield of infectious Tacaribe virus was higher when the inoculum contained a low proportion of DIP. In independent experiments performed with different inocula, a direct correlation was found between the ratio of standard viruses to DIP in the inoculum and the ability to induce inhibition of both Ca²⁺ and Na⁺/K⁺ ATPase activities (results not shown).

In summary, in the present report it is demonstrated for the first time that infection by an arenavirus may induce inhibition of the activities of the host cell Na⁺/K⁺ and Ca²⁺ pumps. This effect of virus infection is related to the replication of standard viruses and is modulated by DIP.

It is well known that Ca²⁺ works as a second messenger for certain cellular signals. To accomplish such a role, it is necessary that cells maintain a large gradient of Ca²⁺ across the plasma membrane, and this is mainly achieved by the Ca²⁺ pump (Kretsinger, 1981; Cheung, 1983, 1984). On the other hand, the Na⁺/K⁺ ATPase plays a complementary role by maintaining a gradient of Na⁺ and K⁺ ions across the plasma membrane. This gradient energizes the Na⁺/Ca²⁺ exchange which plays an important function in excitable cells (Baker, 1972). Furthermore, the role of the Na⁺/K⁺ pump in the regulation of membrane potential and cell volume is well known (Glynn, 1985). Therefore virus-mediated alterations of the Ca²⁺ and Na⁺/K⁺ pumps may result in cation imbalances that, according to their importance and the type of cell involved, may lead to structural damage of the cell and/or may induce perturbations of cellular functions, among which the specialized functions of secretory and excitable cells should be particularly sensitive (Cheung, 1983, 1984). In this regard the reports of Oldstone and coworkers should be mentioned (Oldstone et al., 1984a, b; Valsamakis et al., 1987); they demonstrated that LCM virus replication in pituitary and β cells of the islets of Langerhans is associated with alterations of specialized cellular functions without producing cell damage.

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