Na and K Changes in Animal Virus-infected HeLa Cells

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SUMMARY

Na-K changes and cytotoxic effects were monitored in HeLa cells infected with encephalomyocarditis (EMC) virus, human rhinovirus or vesicular stomatitis virus (VSV). Whereas in all three cases Na-K changes followed viral inhibition of cellular protein synthesis, such changes preceded trypan blue staining of rhinovirus-infected or VSV-infected cells and paralleled trypan blue staining of EMC virus-infected cells. In each case a progressive reduction in cellular electrical volume was associated with Na-K changes. K leakage from EMC virus- or rhinovirus-infected cells clearly exceeded Na gain by these cells. These findings are basically similar to those previously reported for poliovirus-infected HeLa cells. It is concluded that Na-K changes induced by picornaviruses or VSV do not trigger shut-off of HeLa cell protein synthesis, but may contribute to late manifestations of cytotoxicity.

According to one hypothesis, virus-induced enhancement in cellular Na concentration mediates picornavirus shut-off of host protein synthesis (Carrasco & Smith, 1976). This hypothesis is not supported by results obtained with poliovirus-infected HeLa cells (Nair et al., 1979; Nair, 1981) or mengovirus-infected mouse ascites tumour cells (Egberts et al., 1977). In both cases Na accumulation by infected cells follows virus-induced shut-off of cellular protein synthesis. A similar situation exists in vaccinia virus-infected HeLa cells (Norrie et al., 1982) and may also exist in vesicular stomatitis virus (VSV)-infected L-cells as judged from the nature of K changes in this system (Francoeur & Stanners, 1978). In poliovirus-infected HeLa cells Na-K changes correlate with virus-induced late morphological alterations (Nair et al., 1979; Nair, 1981). On the other hand, in L-cells infected with encephalomyocarditis (EMC) virus (Ramabhadran & Thach, 1981), and in chick embryo cells infected with Sindbis virus (Garry et al., 1979), a correlation between viral inhibition of cellular protein synthesis and monovalent cation changes has been reported.

Since different cell types, viruses and conditions of infection were used in the various studies mentioned above, it was felt that a comparative study of Na-K changes induced by different viruses under similar conditions in the same cell type would be helpful in evaluating the involvement of cation changes in virus cytotoxicity. Therefore, Na-K changes were measured in HeLa cells infected under conditions of high multiplicity of infection, with EMC virus, human rhinovirus type 14, or VSV, in terms of cumulative uptake of $^{22}\text{Na}$, and Na-K contents. The cation changes were correlated with inhibition of cellular protein synthesis, changes in cellular electrical volume, and trypan blue staining. The results did not implicate monovalent cation changes in the initiation of shut-off induced by the two picornaviruses or VSV in HeLa cells. On the other hand the results did suggest a correlation between monovalent cation changes and a reduction in cellular electrical volume induced by these viruses.

In EMC virus-infected HeLa cells, a moderate inhibition of host protein synthesis was evident by 2 h post-infection whereas an increase in $^{22}\text{Na}$ uptake did not occur until 3.5 h (Fig. 1a). The increase in $^{22}\text{Na}$ uptake was associated with a twofold increase in cellular Na content, an even greater reduction in cellular K content (Fig. 1a) and a decrease in cellular electrical volume (Fig. 1b). The fraction of cells that could be stained with trypan blue remained insignifi-
Fig. 1. Na–K changes and cytotoxic effects in animal virus-infected HeLa cells. Monolayer cultures of HeLa cells were infected with EMC virus (a, b), human rhinovirus type 14 (c, d) or VSV (e, f) at 250 p.f.u./cell, by incubation at 34 °C for 30 min (EMC virus, rhinovirus) or at 37 °C for 1 h (VSV). To determine cumulative uptake of $^{22}$Na (O; a, c, e), the cultures were incubated with MEM containing 25 mM-HEPES, 2% calf serum and antibiotics (HEPES–MEM), in the presence of 1 μCi/ml $^{22}$NaCl (carrier-free, New England Nuclear). At the intervals indicated, pairs of cultures were rapidly washed with cold phosphate-buffered saline (PBS) and lysed with water. Protein content and the radioactivity of the lysates were assayed by the Lowry et al. (1951) procedure and by scintillation counting (Nair et al., 1979), respectively. To measure Na–K contents [numbers in panels (a), (c) and (e) expressed as nmol/mg protein], the cultures were incubated with HEPES-MEM. At the intervals shown, the cultures were rapidly washed with warm isotonic sucrose and lysed with deionized water. The lysates were assayed for Na–K content by flame photometry (Nair, 1981) and for protein content as mentioned above. To determine rates of protein synthesis (O), EMC virus-infected (a) or rhinovirus-infected (c) cultures were incubated for 20 min with HEPES–MEM containing 5 to 10 μCi/ml $[^3]$H]leucine, at the indicated intervals after infection. After rapid washing of cultures with cold PBS, acid-soluble radioactivity was removed from cultures by two, 5 min rounds of extraction with warm 5% TCA. The radioactivity in the extracts was determined by scintillation counting. The acid-insoluble radioactivity in the cultures was solubilized with alkali and assayed after neutralization, by scintillation counting. Label incorporation at each time point was expressed as (acid-insoluble c.p.m./acid-soluble c.p.m.) × 100 and the values obtained with infected cells are expressed as percentages of the corresponding values obtained with control cells. A Coulter counter with a channelizer attachment was used to obtain relative volume profiles of cells (Nair, 1981) at the following times after infection. (b) O, 1 h; O, 3–5 h; O, 4 h. (d) O, 4–5 h; O, 7 h. (f) O, 1 h; O, 4 h. For this purpose, cell suspensions were prepared by trypsinization of rhinovirus-infected (d) or VSV-infected (f) monolayer cultures. To monitor volume changes following EMC virus infection (b), suspension cultures of HeLa S3 cells were employed, since during the later stages of infection with EMC virus, monolayer cultures of the HeLa cell line used in this study underwent massive lysis upon trypsin treatment. A leftward shift of the profile indicates decrease in cellular electrical volume.
cant at early intervals and increased from 10% at 3 h to 25% at 3.5 h and nearly 100% at 4 h post-infection.

Similar results were obtained with HeLa cells infected with human rhinovirus type 14. In these, shut-off of host protein synthesis could be observed by 3 h, whereas a pronounced increase in $^{22}$Na uptake was not evident until about 5 h post-infection (Fig. 1c). Once again the increase in $^{22}$Na uptake was associated with a doubling of cellular Na content, a decrease in cellular K content (Fig. 1c) and an apparent reduction in cellular volume (Fig. 1d). The fraction of cells that could be stained with trypan blue increased from 0% at 5 h to 10% at 7 h and 90% at 11 h post-infection, suggesting that the increase in $^{22}$Na uptake preceded the increase in the trypan blue-staining fraction of cells. The apparent volume decrease was absent at 4.5 h after infection and earlier intervals when the volume profiles of control cells and infected cells were virtually identical (results not shown).

The monovalent cation changes induced by VSV were basically similar to those induced by EMC virus and rhinovirus. Thus, at 4.5 h post-infection, when cell rounding had already occurred, there was a significant increase in $^{22}$Na uptake, a doubling of cellular Na content, moderate loss of cellular K content (Fig 1e), and a marked decrease in cellular electrical volume (Fig. 1f). The volume decrease was not apparent until about 3.5 h and continued after 4 h post-infection (results not shown). $[^3]$H]Leucine incorporation into acid-insoluble material did not reveal any decrease in the rate of protein synthesis in infected cells between 1 and 4 h post-infection, the rates being 97%, 108%, 103% and 105% of control synthesis at 1, 2, 3 and 4 h respectively. Analysis by SDS–PAGE, on the other hand, indicated that inhibition of cellular protein synthesis had started and virus-specific protein synthesis was well established by 2 h post-infection (results not shown). Therefore, it would appear that the decrease in cellular protein synthesis and increase in viral protein synthesis counterbalanced each other, resulting in a constant overall rate of protein synthesis. As with rhinovirus-infected HeLa cells, Na–K changes in VSV-infected HeLa cells also preceded trypan blue staining; even at 5 h post-infection when significant Na–K changes and cell rounding had already occurred, only about 5% of VSV-infected cells retained the dye. However, it appeared that VSV-induced cation changes in HeLa cells also correlated with late morphological changes rather than with the shut-off of cellular protein synthesis. Francoeur & Stanners (1978) reached a similar conclusion on VSV-induced K changes in L-cells.

The results of this study do not support the notion that an increase in Na influx was responsible for protein synthesis inhibition in HeLa cells by EMC virus, human rhinovirus or VSV since Na increase in cells infected with these viruses followed viral inhibition of host protein synthesis. While Na–K changes could not have initiated host inhibition, it is possible that such changes promoted viral gene expression and host shut-off by these viruses. Using lower multiplicities of infection than those employed in this study, other investigators have demonstrated a correlation between virus-induced monovalent cation changes and inhibition of cellular protein synthesis in VSV or Sindbis virus-infected chick embryo fibroblasts (Garry et al., 1979; Garry & Waite, 1979) and in EMC virus-infected L cells (Ramabhadran & Thach, 1981). Perhaps Na–K changes are a cause of host shut-off in some cell types but not in others, and/or when the ratio of infecting virus to cell is relatively low. The results obtained in this study on HeLa cells infected with EMC virus, human rhinovirus or VSV are similar to those previously obtained with poliovirus-infected HeLa cells (Nair et al., 1979; Nair, 1981). In all four cases Na–K changes followed viral inhibition of cellular protein synthesis and coincided with late morphological changes.

Volume measurements with a Coulter counter are subject to errors due to changes in the conductivity of cellular membranes (Schaefer et al., 1982) and in the extent to which cells are distorted while passing through the aperture of the instrument. Increased conductivity and/or distortion of cells would erroneously indicate volume decrease. The Na–K changes observed in this study suggest increased conductivity of infected cells, and it is possible that infected cells undergo greater distortion than control cells during electrical volume measurements. Therefore, the shifts observed in Coulter counter volume profiles of infected cells may actually reflect membrane alterations rather than a reduction in cellular volume. The fact that the reduction reported previously in the electrical volume of poliovirus-infected HeLa cells (Nair, 1981) could
not be confirmed by a $^3$H$_2$O partitioning procedure (Schaefer et al., 1982) supports this view. Regardless of whether or not the reduction in electrical volume indicated cell shrinkage, the close correlation observed between this phenomenon and changes in plasma membrane-dependent functions (morphological changes, Na–K changes, etc.) suggests that electrical volume measurement provides a rapid and simple means to monitor the onset of late membrane alterations in HeLa cells infected with picornaviruses and VSV.

K loss from HeLa cells infected with EMC virus, rhinovirus and possibly with VSV exceeded Na gain by these cells. Assuming that similar Na–K changes occur in the tissues of the infected host, excessive leakage of K from infected cells into the extracellular fluids may contribute to the symptoms associated with these viral infections. Trypan blue staining by EMC virus-infected cells closely paralleled cellular Na–K changes making it difficult to suggest that one was the cause of the other. On the other hand, in rhinovirus or VSV-infected HeLa cells, Na–K changes clearly preceded trypan blue staining, meaning that at least in these instances Na–K changes were not a consequence but could have been a cause of loss of cell viability. These observations support the hypothesis that virus-induced permeability changes in cellular plasma membrane may be involved in the pathological effects of certain viral infections (Pasternak & Micklem, 1981).

We have partially extended these observations to HeLa cells infected with adenovirus type 2 and to CV-1 monkey kidney cells infected with herpes simplex virus type 1. There was no evidence that either of these viruses induced significant Na–K changes in infected cells during one-step growth (C. N. Nair, unpublished observations). The absence of monovalent cation changes in association with herpes simplex virus-induced inhibition of protein synthesis in Vero cells has been reported by Hackstadt & Mallavia (1982). Thus, from the results of this and previous studies, it would appear that Na–K changes do not occur in infected cells soon enough to initiate inhibition of host protein synthesis caused by several viruses, and that such changes are not invariably associated with toxic effects produced in cultured cells by all animal viruses.

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REFERENCES


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