Reversed Polarity in Transmembrane Potentials of Cells Infected with Herpesviruses (36320)

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The first phase in the infection of a cell by a virus (attachment) is electrostatic in character (1). Although the electrical charge of several viruses (2) and of the surface of a variety of cells (3, 4) has been recorded previously, the charge on the surface of cells infected with viruses has received little attention. It was of particular interest to determine the transmembrane potential of herpesvirus-infected cells by microelectrode techniques (5, 6), since membrane changes have been shown to occur after infection with members of this virus group by a variety of other methods (7–10).

This report presents results of recordings of the transmembrane potentials of cells infected *in vitro* with herpes simplex virus type 1 or type 2 or with cytomegalovirus. For comparison, results obtained with cells infected with other viruses—adenovirus type 2, Coxsackievirus B_5, or measles virus—are included.

Methods. HEp-2 cells (originated from cancer of the larynx), grown in 32-oz bottles in Eagle's MEM with 2% calf serum, were infected with herpes simplex virus (HSV) type 1 (strain VR_3) or with HSV type 2 (strain MS) at a multiplicity of infection of 5–10/cell. The cells were harvested at 4, 8, and 14–24 hr, at which time the cytopathic effect was almost complete. The cells were removed with glass beads to avoid digestion by trypsin, washed 3 times in phosphate buffered saline [(PBS); pH 7.2], and pelleted in small tubes. The volume of the cells was approximately 0.5 ml. Lung human fibroblast tissue culture cells were infected with both viruses in a similar manner, but these cells were harvested only when the cytopathic effect was almost complete. Noninfected HEp-2 and fibroblast cells were also processed similarly. In one experiment, HEp-2 cells were stored in PBS for 24 hr at 4° prior to use; and, in another study, HEp-2 cells were boiled for 5 min.

HEp-2 cells were also infected with Coxsackievirus B_5, adenovirus type 2, or measles virus (Edmonson strain) and fibroblast cells were infected with cytomegalovirus (CMV strain AD-169). Cells infected with these four viruses were harvested when the cytopathic effect was almost complete.

Microelectrode recording was performed with glass micropipettes filled with 3 *M* KCl (11). The diameter of the tips was less than 1.0 μ, resistances measured from 10 to 30 MΩ and tip potentials were less than 5 mVolts before compensation. The microelectrode was connected to the cathode follower of a battery-operated dc amplifier (Picometric amplifier 181, Instrumentation Laboratory, Inc., Portland, OR) with grid current less than 10^{-11} A, which in turn was led to an oscilloscope (Rm 565, Tetronix, Inc., Portland, OR). Permanent records were obtained by photography of the screen of the oscilloscope (Oscilloscope camera C-27, Tektronix, Inc., Portland, OR) as the microelectrode was advanced through the layer of cells with the use of a hydraulic microdrive (David Kopf Instruments, Tujunga, CA). A chlorided silver wire of the same diameter as that used in the shaft of the electrode was placed in the medium (PBS) to serve as a reference electrode. Only those impalements which maintained a steady potential for 30 sec, with no change in electrode resistance after impalement, are included in the analysis of data.

Results. The initial recordings from nonin-

TABLE I. Transmembrane Potentials of HEp-2 and/or Human Lung Fibroblast Cells Noninfected or Infected with Herpes Simplex Virus (HSV) Types 1 or 2, Cytomegalovirus, Measles Virus, Coxsackievirus B_5 and Adenovirus Type 2.

Cells	Virus used for infection	Time after infection (hr)	No. of impalements	Av ± SD transmembrane potential (mV)
A. HEp-2 cells	(1) a. None	—	54	−20.7 ± 5.2
	b. None	(after 24 hr at 4°)	13	−19.9 ± 6.4
	c. None	(after boiling for 5 min)	15	0
	(2) a. HSV-type 2	4	9	−14.4 ± 3.1
	b. HSV-type 2	8	21	+4.5 ± 1.2
	c. HSV-type 2	14–24	48	+12.4 ± 5.9
	(3) a. HSV-type 1	4	16	−11.7 ± 3.2
	b. HSV-type 1	8	17	+4.5 ± 1.9
	c. HSV-type 1	14–24	38	+8.2 ± 3.3
	(4) Measles virus	96	28	+6.8 ± 2.3
	(5) Coxsackievirus B_5	72	26	−14.5 ± 4.2
	(6) Adenovirus type 2	48	22	−19.0 ± 2.7
B. Human lung fibroblast cells	(1) None	—	26	−21.1 ± 3.4
	(2) HSV-type 2	24	22	+12.9 ± 3.8
	(3) HSV-type 1	24	17	+7.2 ± 2.1
	(4) Cytomegalovirus	144	15	+6.8 ± 1.8

fected HEp-2 and human fibroblast cells (Table I) revealed a transmembrane potential of −20.7 ± 5.2 and −21.1 ± 3.4 mV, respectively. After infection of the cells with HSV types 1 or 2, the polarity shifted to being less negative 4 hr after infection, increasing to positive polarity in the 8 hr and 14–24 hr specimens. The magnitude of the positive potential in either the HEp-2 or fibroblast cells infected for the 14–24 hr period was slightly higher in cells infected with HSV type 2 than with HSV type 1 (Table I). Membrane potentials from cells refrigerated for 24 hr were negative; potentials from HEp-2 cells, which were boiled, measured zero.

Membrane potentials recorded from HEp-2 cells infected with either Coxsackievirus B_5 or adenovirus type 2 all displayed negative polarity, the mean value being −14.5 ± 4.2 mV for cells infected with the Coxsackievirus and −19.0 ± 2.7 mV for cells infected with the adenovirus (Table I). Cells infected with cytomegalovirus or measles virus demonstrated positive polarity, the mean value being +6.8 ± 1.8 mV for cells infected with cytomegalovirus and +6.8 ± 2.3 mV for cells infected with measles virus.

Discussion. The negative transmembrane potentials observed with normal (fibroblast) and malignant (HEp-2) cells and the gradual increase in polarity toward positivity correlating with increased time after infection wth herpes simplex virus types 1 or 2 (Table I) suggest that the reversal of polarity was a result of infection with these viruses. The observation that uninfected cells, refrigerated for 24 hr, still demonstrated negative transmembrane potentials and that boiled cells displayed zero potential indicate that cell death alone could not account for the reversal of polarity. This conclusion is further supported by the internal negativity displayed by HEp-2 cells infected with two other viruses, adenovirus type 2, and Coxsackievirus B_5.

The reversed polarity in transmembrane potential observed in cells infected with cytomegalovirus or measles virus, or with either of the two types of HSV (Table I) contrasts with the internal negativity maintained by cells infected with adenovirus type 2 or with Coxsackievirus B_5. Since the 3 herpesviruses and measles virus are enveloped, while

adeno- and Coxsackievirus are nonenveloped, it is possible that reversed polarity will be found to be associated primarily with enveloped viruses. Further studies with other enveloped and nonenveloped viruses should allow confirmation of this hypothesis.

The results on the membrane potential of uninfected HEp-2 cells agree quantitatively with those described by Hause *et al.* (12). We were, however, unable to demonstrate the small positive surface prepotentials noted by these workers in their recordings from uninfected HEp-2 cells in monolayers on glass slides. Although the recordings reported here have been made with pelleted cells, preliminary results using Hause's technique have confirmed the reversed polarity in transmembrane potential of HEp-2 cells infected with herpes simplex viruses.

Our present working hypothesis to explain the phenomenon of a virus-infected cell shifting to positive polarity is that, as the cell membrane is altered, there is enhanced movement of sodium ions into the cell and, hence, of water. The ballooning of herpes-infected cells, first described by Unna in late 19th century, could be a result of this process. A marked change in membrane permeability of herpes-infected cells has already been demonstrated by other workers, who reported on the leakage from infected cells of large molecules, such as proteins (13). Flux studies to determine the nature of the ionic distribution across the membrane of herpes-infected cells would allow further testing of this hypothesis. In addition, similar studies, as those presented here, on oncogenic and nononcogenic viruses should provide important information regarding the membrane alterations induced by such viruses.

Summary. Transmembrane potentials were recorded by electrophysiological techniques in HEp-2 tissue culture cells after infection with herpes simplex virus types 1 or 2, measles virus, Coxsackievirus B_5, or adenovirus type 2, and in human fibroblast cells infected with herpes simplex virus type 1 or type 2 or with cytomegalovirus. Non-infected HEp-2 or fibroblast cells displayed negative polarity; whereas cells infected with types 1 or 2 herpes simplex viruses, cytomegalovirus, or measles virus displayed a shift to positive polarity. HEp-2 cells infected with Coxsackievirus B_5 or adenovirus type 2 retained their internal negativity. These results suggest that further application of similar electrophysiological techniques might prove useful in the study of cell membrane changes induced by enveloped or oncogenic viruses.

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