Short Communications

Virally Mediated Membrane Changes: Inverse Effects on Transport and Diffusion

By CHARLES A. PASTERNAK and KINGSLEY J. MICKLEM

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

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Sendai virus modifies the cell surface in two ways: (a) by inhibiting facilitated transport and (b) by enhancing passive diffusion.

The observation (Pasternak & Micklem, 1973) that Sendai virus causes leakage of metabolites from a variety (Pasternak & Micklem, 1974) of cells and at the same time inhibits uptake seems at first sight paradoxical. This is because the metabolites that leak out include phosphorylated derivatives, which presumably leave cells by passive diffusion. Since passive diffusion is, by definition, a symmetrical process, stimulation of leakage should be accompanied by stimulation of uptake, provided the latter is also by passive diffusion. Because the original uptake experiments were carried out with low concentrations of substrates such as choline, deoxyglucose and aminoisobutyrate (Pasternak & Micklem,

1973), carrier-mediated transport is likely to have predominated over passive entry. We have now studied the uptake of these and other substrates over a range of concentrations in order to separate the effects of virus on passive diffusion (dominant at high concentration) and on facilitated transport (dominant at low concentration); the predicted increase in uptake at high substrate concentration was observed.

Methods and materials

Lettree cells were exposed to Sendai virus and leak-out of previously accumulated radioisotope was measured as described by Pasternak & Micklem

Fig. 1. Effect of Sendai virus on uptake of choline

Lettree cells (2×10^6 /ml) with (●) or without (○) virus (200 haemagglutinin units/ml) in phosphate-buffered saline were incubated at 37°C with [Me -³H]choline (0.6–5 μCi/ml) at various concentrations and uptake was measured as described under 'Methods and materials'. (a) 0.125 mM; (b) 0.5 mM; (c) 5 mM; (d) 20 mM.

Table 1. *Virally mediated membrane changes*

Substrate	Concentration (mM)	Effect of virus	Predominating process
Choline	<0.5	Inhibition of entry	Carrier-mediated transport
2-Deoxyglucose	<3		
2-Aminoisobutyrate	<20		
Choline	>0.5	Stimulation of entry	Passive diffusion
2-Deoxyglucose	>3		
2-Aminoisobutyrate	>20		
Methyl α -glucoside	Any		

(1973, 1974). Uptake was measured by simultaneously adding virus (under non-agglutinating conditions; Pasternak & Micklem, 1974) or phosphate-buffered saline and radioisotope to cell suspensions in conical centrifuge tubes. After appropriate intervals of time the suspensions were diluted five-fold with phosphate-buffered saline, centrifuged, the walls of the tube were dried with soft tissue paper and the radioactivity in the pellet was measured (Pasternak & Micklem, 1973).

[Me- ^3H]Choline chloride (1 Ci/mmol) and methyl α -[U- ^{14}C]glucoside (3 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and were diluted with unlabelled material as required.

Results and discussion

Fig. 1 shows how Sendai virus modifies the rate of uptake of choline into Lettree cells. Below 0.5 mM-choline in the medium virus is inhibitory, whereas above 0.5 mM-choline the virus stimulates entry. Similar results have been obtained with HeLa cells. For the uptake of deoxyglucose and aminoisobutyrate, the respective 'cross-over' points (i.e. concentration at which the rate of uptake in the presence of virus is the same as in the control) are approx. 3 and 20 mM. Accumulated isotope tends to leak out again after some minutes; this effect is particularly marked in the presence of virus and at high concentrations of substrate. It is pertinent to note that K_m values quoted for the entry of choline, e.g. 4–7 μM (Plagemann, 1971), deoxyglucose, e.g. 2 mM (Hatanaka *et al.*, 1970; Renner *et al.*, 1972; Kletzien & Perdue, 1973) or less (Mizel, 1973), and aminoisobutyrate, e.g. 1–2 mM (Foster & Pardee, 1969; Isselbacher, 1972), are in each case below or near the 'cross-over' point. (At concentrations far below the K_m , the contribution of passive diffusion to uptake again becomes significant and a reversal of the effect depicted in Fig. 1 is to be expected; this has been observed in some instances.) Whether the increased rate of diffusion caused by Sendai virus is in any way related to the permanently altered uptake observed in virally transformed cells (e.g. Foster & Pardee, 1969) remains to be seen.

In the case of substances that are transported by

passive diffusion alone, the rates of exit and entry at equivalent intra- and extra-cellular concentrations, in the presence and absence of virus, should be the same. Methyl α -glucoside behaves in just this manner. Thus the rate of entry of methyl α -[U- ^{14}C]glucoside (2.5 $\mu\text{Ci/ml}$; 0.5 mM) into Lettree cells ($1.5 \times 10^7/\text{ml}$) at 37°C was found to be 40 nmol/min per ml of cell water without virus and 150 nmol/min per ml of cell water in the presence of virus (400 haemagglutinin units/ml). The rate of exit from cells ($2.1 \times 10^7/\text{ml}$) which had been prelabelled for 2 h with methyl α -[U- ^{14}C]glucoside (2 $\mu\text{Ci/ml}$; 2 mM) and washed (internal concentration of methyl α -glucoside approx. 0.5 mM) was 23 nmol/min per ml of cell water without virus and 125 nmol/min per ml of cell water in the presence of virus (1600 haemagglutinin units/ml). Although one might expect that phosphorylcholine would follow a similar pattern, we have as yet been unable to demonstrate significant uptake, despite extensive leak-out in the presence of virus.

One may conclude that Sendai virus modifies the cell surface in two distinct ways (Table 1). The mechanisms underlying these changes are not yet clear. So far as the increase in passive diffusion is concerned, the observation that Sendai virus causes a clustering of particles within the plane of the plasma membrane (Bächi *et al.*, 1973) is pertinent. For a similar clustering of particles has been reported to accompany the formation of intercellular junctions in cultured cells, and these, too, allow the passage of phosphorylated metabolites out of (and into) cells (Pitts, 1975). Finally the inverse nature (Table 1) of the permeability changes here reported enable one to distinguish between facilitated transport and passive diffusion in a novel and rather simple manner.

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