Volume 234, number 2, 449–450 FEB 06048 July 1988

Intracellular pH and cell adhesion to solid substrate

L.B. Margolis, I.A. Rozovskaja and E. Cragoe*

Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry of Moscow State University, Moscow 118899, USSR and **Merck, Sharp and Dohme Research Laboratories, West Point, NY, USA*

Received 5 May 1988

It was shown that activation of the Na^+/H^+ antiporter resulting in an increase of intracellular pH (pH_i) by 0.2–0.3 is a necessary stage of cell stimulation by soluble growth factors. Solid substrate can also be formally regarded as a growth factor since adhesion stimulates proliferation of various cell types. In the present study we have found that the attachment of mouse embryo fibroblasts to solid substrate is followed by an increase of pH_i by approx. 0.3 units. pH shift occurs after the cell attaches to the substrate and is obligatory for cell spreading. The evidence for Na^+/H^+ antiporter involvement in the increase of pH_i in substrate-attached cells is presented. It is suggested that signals for cell proliferation by chemical (soluble ligands) and physical (solid substrate) growth factors are transmitted similarly.

Na^+/H^+ antiporter; Cell adhesion; Amiloride derivative

1. INTRODUCTION

Recently activation of the Na^+/H^+ antiporter resulting in the alkalinization of the cell interior by 0.2–0.3 pH units was shown to be an essential stage in the stimulation of DNA synthesis by soluble growth factors bound to the cell surface [1–3].

Solid substrate can also be formally regarded as a growth factor since adhesion to the substrate stimulates proliferation of many cell types. Malignant transformation brings about the release of cell proliferation from its substrate dependence.

We have found that the attachment of mouse embryo fibroblasts to solid substrate is followed by an increase of intracellular pH (pH_i) by 0.2–0.3 units. The pH shift is mediated by the Na^+/H^+ antiporter and is obligatory for the development of the cell adhesion.

2. MATERIALS AND METHODS

The following media were used for the experiments with cells: (i) Dulbecco's modified Eagle's medium; (ii) phosphate buffered solution (PBS); (iii) Na^+-physiological solution consisting of 140 mM NaCl and 20 mM Tris buffer; (iv) Na^+-free physiological solution consisting of 140 mM choline chloride and 20 mM Tris buffer.

Amiloride derivative, 5-(*N*-methyl-*N*-isobutyl)amiloride, highly selective in inhibiting Na^+/H^+ antiporter was synthesized by one of the authors (E.C.) and dissolved in DMSO. Nigericin (Calbiochem) was dissolved in ethanol. Mouse embryo fibroblasts were isolated as described earlier [4].

For the experiments cell suspension was placed in flasks above the coverslip. The pH_i of individual cells was measured 20–30 min later. Non-adhesive substrate was prepared by covering the coverslip with egg phosphatidylcholine as described earlier [4,5].

For measuring intracellular pH we have used the pH-sensitive fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF) from Molecular Probes, as described in [6]. The calibration curve was obtained by the nigericin-potassium method [7]. In every experiment the pH_i of 30–50 cells was measured and the mean value (± SE) was calculated.

Correspondence address: L.B. Margolis, Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry of Moscow State University, Moscow 118899, USSR

3. RESULTS AND DISCUSSION

Adhesion is a complex chain of cellular reactions initiated by the contact of plasma membrane with a solid surface. This includes the attachments of initially round cells by short (1–2 μm) processes,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

Table 1

Adhesion-induced increase of intracellular pH

	Monolayer (5 h)	Suspension (15 min)	40 min on adhesive glass substrate	40 min on non-adhesive lipid substrate
Intracellular pH	7.55 ± 0.02	7.30 ± 0.04	7.50 ± 0.02	7.30 ± 0.01

developing of longer pseudopodia and lamellae and finally cell spreading on the substrate.

When the suspended cells adhere to the solid substrate in the serum-supplemented growth media or PBS, an increase of pH_i by 0.2–0.3 units is observed (table 1). The pH shift is reversible: the detachment of the substrate-attached cells either by trypsin or by EDTA results in a decrease of pH_i back to its initial value (table 1).

What is the particular morphological stage of cell adhesion that is associated with an increase of pH_i. It seems that the increase of pH_i occurs during the early stages of cell spreading. Indeed, a pH shift was observed even in the substrate-attached cells which under phase-contrast microscopy seemed either completely spherical or to bear only short processes.

However, mere physical contact with the solid substrate is not sufficient for the development of a pH shift. When non-adhesive phospholipid substrate was used [5] no increase of pH_i was observed upon cell contact with such a surface (fig.1).

Thus the increase of pH_i occurs after the initial attachment of the cell to the substrate but before the development of long filopodia and lamellae.

To understand whether the Na^+/H^+ antiporter is involved in the increase of pH_i associated with cell attachment to a solid substrate, we studied the whole process either in an Na^+-free medium or in the presence of an amiloride analogue.

In a typical experiment where cells were allowed to attach to the glass surface in an Na^+-free medium (pH 7.4), the pH value remains 6.9 ± 0.04 for at least an hour. However, when the medium was changed for that containing Na^+, the pH_i value of the attached cells increased immediately by 0.2 ± 0.04 units. In contrast to the substrate-attached cells, the pH of suspended cells did not change after the Na^+-free medium was substituted by that containing Na^+. It remained 6.9 ± 0.04.

The Na^+-stimulated increase of pH_i was found to be sensitive to the amiloride derivative 5 μm. Addition of this substance 10 min before Na^+ completely prevents the increase in pH value of substrate-attached cells. Amiloride treatment of cells suspended in the growth medium decreases their pH_i value by approx. 0.3 units and prevents the increase of pH after cell attachment. Cell spreading on the substrate was also inhibited: the attached cells remain spherical (observed using phase-contrast microscopy).

Thus it seems that the increase in pH in our experiments is mediated by the Na^+/H^+ antiporter. Its stimulation upon cell attachment to the solid substrate seems to be an obligatory prerequisite stage of cell spreading. It remains to be studied what the key intracellular reaction(s) in the complex phenomenon of cell spreading is (are) that is (are) affected by the increase in pH_i. Perhaps it is the pH gradient rather than the absolute value of pH_i that is critical to the cell.

Anyway the data presented above show the similarity of early stages in the transmission of a signal for cell activation by chemical (soluble ligands bound to the cell surface) and physical (cell binding to solid substrate) growth factors.

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