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Acidification of the interior of Ehrlich ascites tumor cells by nigericin inhibits DNA synthesis

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In Ehrlich ascites carcinoma cells, acidification of the cytoplasm down to pH 6.2–6.3 arrests DNA synthesis. Such acidification can be obtained by decreasing the pH outside the cell or, alternatively, by addition of a micromolar concentration of the K^+/H^+ antiporter nigericin. Thus, nigericin may be regarded as a new type of cytostatic, the effect of which is mediated by alteration of the intracellular pH.

intracellular pH; K^+/H^+ antiporter; DNA synthesis; (Ehrlich ascites tumor cell)

1. INTRODUCTION

The activation of DNA synthesis upon a rise in intracellular pH (pH_i) has been well documented for several cell types [1–3]. Although it is now disputed as to whether a pH shift for blood mononuclear cells is essential [4], at least for fibroblasts entry into the S-phase can be prevented by acidification of the cell interior as a result of a decrease in the pH of the outer media (pH_o) down to pH 6.9 [5,6]. In contrast to normal cells cancerous tissue somehow continues to grow even in a highly acidic environment. Ehrlich ascites carcinoma may be an example of such a tumor.

We have investigated the pH dependence of DNA synthesis in Ehrlich ascites tumor cells which normally proliferate in ascitic fluids at pH 6.9. It is demonstrated that the pH optimum of DNA synthesis in these cells is rather broad: they stop synthesizing DNA at pH_i 6.3. DNA synthesis can be inhibited at the physiological outer pH by the exogenous K^+/H^+ antiporter nigericin, which decreases the intracellular pH.

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2. EXPERIMENTAL

Ehrlich ascites carcinoma cells were used on day 6 after i.p. implantation. During experiments cells at a concentration of 3×10^7 cells/ml were incubated at 37°C in 199 medium buffered with 50 mM Tris-HCl (pH 7.0–7.8) or 50 mM Mes-NaOH (pH 6.2–6.9). The flasks containing the cells were continuously shaken. Nigericin (Calbiochem) was added to the cell suspension at various concentrations. [^{14}C]Thymidine (20 Bq) was added 10 min later. Aliquots of the cell suspension were sedimented immediately, 15 and 30 min after addition of the radioactive precursor. The radioactivity of the acid-insoluble fraction of these cells was evaluated in a Mark III liquid scintillation counter (Nuclear Chicago).

The intracellular pH was evaluated using the pH-sensitive dyes fluorescein diacetate (Fluka) or 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (acetoxymethyl ester, Molecular Probes), according to the procedure developed by Tsien et al. [7]. The fluorescence intensity at two wavelengths

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was measured with an Opton fluorescence microscope, equipped with and FML-2 microphotometer (Lomo).

The state of mitochondria in ascites cells was evaluated by rhodamine 123 staining. The dye (10 μg/ml) was added to the medium for 10 min. The cells were then washed with rhodamine-free solution and observed with an Opton III fluorescence photomicroscope.

3. RESULTS AND DISCUSSION

The ability of Ehrlich ascites carcinoma cells to synthesize DNA at an outer pH of 6.9 could be explained by two alternative hypotheses: (i) the endogenous Na^+/H^+ antiporters are so active upon stimulation that they maintain the internal pH at 7.4 which is necessary for DNA synthesis, e.g. in fibroblasts [5,6]; (ii) the synthesizing machinery of these cells is adapted to low pH.

Our pH_i measurements as well as other data [8] demonstrate that the latter possibility takes place: at pH_o 6.9 maintained in ascitic fluid, pH_i proved to be equal to 6.6–6.7.

We have measured the incorporation of [^{14}C]-thymidine into the acid-insoluble cell fraction at various intracellular pH values. Different pH_i values were achieved by varying the pH of the outer media. During experiments cells were kept in stoppered flasks without aeration in 199 media.

Table 1

Intracellular pH of Ehrlich ascites carcinoma cells

pH of the outer media	Intracellular pH	
	Control	Treated with 10^{-5} M nigericin
6.9	6.60 ± 0.05	6.30 ± 0.07
7.8	6.90 ± 0.06	6.60 ± 0.05

These conditions potentiate glycolysis. Using low pH outer media we have obtained pH_i as low as 6.3. The highest pH_i was 7.1, a value which is maintained at pH_o 7.8. At higher pH_o the cells begin to aggregate into dense clumps.

Thus, the pH_i dependence of DNA synthesis studied in the range pH 6.3–7.2 is shown in fig.1. It is clear from this figure that the pH dependence of DNA synthesis in Ehrlich ascites tumor cells is shifted to a more acidic region compared with that in other cells [2,3]. In these cells DNA synthesis normally proceeds when the pH of the cytoplasm is 6.6–7.0. At lower pH_i it is partly inhibited and is arrested at 6.3.

Further experiments showed that acidification of the cell interior can be achieved without changing pH_o. This was done by addition of the exogenous K^+/H^+ antiporter nigericin. Although the gradients of these two ions across the plasma membrane are unidirectional, under physiological con-

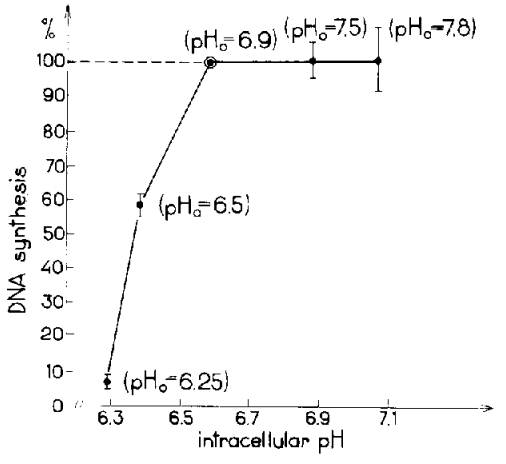

Fig.1. Dependence of DNA synthesis on intracellular pH. pH_i and DNA synthesis were measured at the pH_o values indicated. The level of DNA synthesis at the physiological pH_o 6.9 was regarded as 100%.

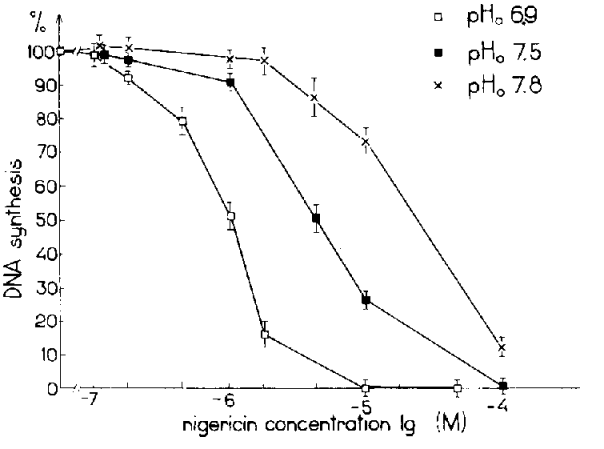

Fig.2. Inhibition of DNA synthesis by nigericin at various pH_o values. The level of DNA synthesis at the physiological pH_o 6.9 was regarded as 100%.

ditions the $[K^+]$ gradient is two orders higher than $[H^+]$ gradient. This means that nigericin should import H^+ into the cytoplasm and export K^+ out of the cell.

The experiments with nigericin confirmed this suggestion. Addition of the antibiotic acidifies the cell interior (table 1). It is also shown that the decrease of pH_i by nigericin inhibits DNA synthesis (fig.2). This inhibition is dependent on the outer pH. When the pH of the outer media was 7.8, DNA synthesis was arrested by 10^{-4} M nigericin. At pH_o 7.0, a similar effect was obtained when 5×10^{-6} M nigericin was added.

As can be seen in table 1 and fig.2, DNA synthesis does not change until pH_i exceeds 6.5. When pH_o and the nigericin concentration are such that pH_i decreases to 6.3, synthesis is blocked. The effect of nigericin resembles that of amiloride (an inhibitor of endogenous Na^+/H^+ antiporters), which also shifts down pH_i and inhibits DNA synthesis [9,10]. However, in the experiments with nigericin the decrease of intracellular K^+ could contribute to the inhibition of DNA synthesis. As reported, there is a threshold inner K^+ level in the cell (about 20 mM) below which DNA cannot be synthesized [11].

Control experiments have shown that the results obtained reflect the decrease in DNA synthesis but not the inhibition of [^{14}C]thymidine entry into the cells.

At all concentrations tested, nigericin did not cause any morphological alterations of cells, as observed by phase contrast microscopy. The mitochondrial membrane potential, as tested with rhodamine 123 staining, was similar in nigericin-treated and control cells. Thus, the inhibition of DNA synthesis is not mediated by mitochondrial uncoupling [12].

Our preliminary results show that nigericin inhibits DNA synthesis in monolayer cultures of fibroblast and epithelial cell lines. In these cultures, nigericin also did not alter cell morphology. Its effect was reversible. DNA synthesis reversed to normal values 6 h after changing the media.

Thus, nigericin can be regarded as a new type of cytostatic. Its effect is potentiated in low pH media. Since this condition is maintained in many tumors with high glycolytic activity, nigericin may prove to be a potent in vivo cytostatic.

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