EFFECT OF OXIDATIVE PHOSPHORYLATION UNCOUPLERS ON DEPOSITION OF NEUTRAL RED GRANULES IN NORMAL AND TUMOR CELLS

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A study of the action of oxidative phosphorylation uncouplers on interaction between neutral red and cultures of normal mouse fibroblasts and L tumor cells showed that the former, unlike the latter, are still able to form granules of the dye in the presence of $1 \cdot 10^{-4}$ M 2,4-dinitrophenol (DNP). Meanwhile p-trifluoromethoxycarbonylcyanide-phenylhydrazone (FCP), another uncoupler of oxidative phosphorylation, inhibits the deposition of granules of the dye both in L tumor cells and in normal mouse fibroblasts. Accumulation of the dye by normal fibroblasts and by L cells is inhibited by both DNP and FCP in concentrations uncoupling oxidative phosphorylation.

KEY WORDS: mouse embryonic fibroblasts; L cells; oxidative phosphorylation uncouplers; deposition of dye granules.

The dependence of deposition of granules of the basic vital dyes and various therapeutic preparations on the energy metabolism of cells has frequently been examined [2, 3, 6-11]. The main sources of energy for cellular activity are the anaerobic and aerobic breakdown of carbohydrates, i.e., glycolysis and respiration.

Oxygen respiration is the principal more efficient method of energy metabolism of normal cells. The cells of most tumors can carry out glycolysis under aerobic conditions [1], and this aerobic glycolysis is the main pathway of energy production in tumor cells.

The work of Zelenin [2, 3] has shown that granule deposition in both normal and tumor cells utilizes energy liberated during glycolysis.

The object of this investigation was to study how uncouplers of oxidative phosphorylation affect the deposition of neutral red granules in normal and tumor cells.

EXPERIMENTAL METHOD

Primary cultures of normal mouse embryonic fibroblasts and a culture of tumor cells, namely the L strain of mouse fibroblasts, were used as the test objects.

The cells were grown in medium of the following composition: 45% medium No. 199, 45% lactalbumin hydrolyzate, and 10% bovine serum.

Depending on the experimental conditions the cells were seeded into penicillin flasks (2.5 ml of the cell suspension per flask), on the bottom of which coverslips measuring 5 × 22 mm were laid, or into Carrel's flasks (6 ml of suspension per flask). The density of the cell suspension used for seeding was

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TABLE 1. Action of DNP on Deposition of Neutral Red Granules in Normal Fibroblasts and L cells

Concentration of uncoupler (DNP), in M	Primary culture of normal mouse embryonic cells	Culture of L cells
Without DNP	Red granules in perinuclear zone in colorless cytoplasm	Red granules in colorless cytoplasm
$1 \cdot 10^{-5}$	Granules in cells	Granules in cells
$1 \cdot 10^{-4}$	Granules in cells	Diffuse staining of cytoplasm and nucleus, red nucleoli, no granules
$1 \cdot 10^{-3}$	Diffuse staining of cytoplasm and nucleus, no granules	Diffuse staining of cytoplasm and nucleus, no granules

TABLE 2. Action of FCP on Deposition of Neutral Red Granules in Normal Fibroblasts and L Tumor Cells

Concentration of uncoupler (FCP), in M	Primary culture of normal mouse embryonic cells	Culture of L cells
Cells without FCP	Red granules in colorless cytoplasm	Red granules in colorless cytoplasm
$5 \cdot 10^{-7}$	Granules in cells	Granules in cells
$1 \cdot 10^{-6}$–$5 \cdot 10^{-6}$	No granules in cells, diffusely stained nucleolus, nucleus, and cytoplasm	No granules in cells, diffusely stained nucleolus, nucleus, and cytoplasm

150,000 cells/ml for normal fibroblasts and 70,000 cells/ml for L fibroblasts. The cell monolayer was sprinkled with Earle's solution and incubated in the same solution containing 2,4-dinitrophenol (DNP) in a concentration of $1 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, and $1 \cdot 10^{-3}$ M or with p-trifluoromethoxycarbonylcyanide-phenylhydrazone (FCP) in a concentration of $5 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, and $5 \cdot 10^{-6}$ M. The cells were incubated in the medium with the uncouplers for 15 min at 37°C, then stained with a 0.005% solution of neutral red in the presence of the uncouplers for 30 min at 37°C.

The character of staining of the cells was studied in the light micrscope, after which the cell monolayer was rinsed a few times with Earle's solution without the dye and the dye which had entered the cells was extracted by means of 70% acidified alcohol directly from the cell monolayer. The quantity of dye in the extract was determined on the SF-4A spectrophotometer at $\lambda = 565$ nm, the maximum of the absorption spectrum of neutral red in acidified alcohol.

EXPERIMENTAL RESULTS

The writers showed previously [4] that DNP, in concentrations uncoupling oxidative phosphorylation, inhibits granule formation in Ehrlich's ascites carcinoma cells and also affects accumulation of the dye by the cells.

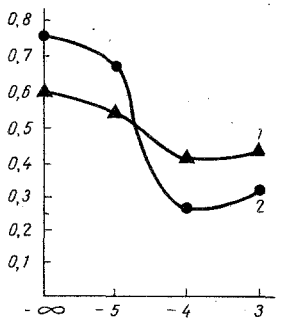

Fig. 1.

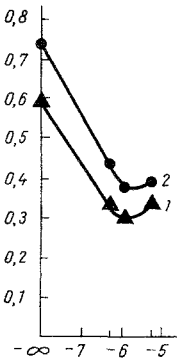

Fig. 2

Fig. 1. Effect of DNP on accumulation of neutral red by normal mouse fibroblasts (1) and by L tumor cells (2). Abscissa, log of DNP concentration (in M); ordinate, amount of dye in cells (in optical density units).

Fig. 2. Effect of FCP on accumulation of neutral red by normal mouse fibroblasts (1) and by L tumor cells (2). Abscissa, log of FCP concentration (in M); ordinate, amount of dye (in optical density units).

The results of these investigations of the effect of DNP on the deposition of dye granules by normal fibroblasts and L cells are given in Table 1. Incubation of the cells with $1 \cdot 10^{-5}$ M DNP did not affect the ability of the cells to deposit granules of the dye. An increase in the DNP concentration to $1 \cdot 10^{-4}$ M (a concentration uncoupling oxidative phosphorylation) led to inhibition of granule deposition only in the L tumor cells. Normal fibroblasts were more resistant to the action of DNP. An increase in the DNP concentration to $1 \cdot 10^{-3}$ M (a concentration inhibiting cellular respiration) prevented the normal fibroblasts from depositing the dye in granules. Uncoupling of oxidative phosphorylation by DNP thus affects granule deposition only by L tumor cells and does not affect the ability of normal fibroblasts to deposit the dye in granules. This result was very unexpected.

The energy metabolism of the tumor cell is known to differ from normal in the greater intensity of its glycolysis [1]. On the other hand, oxidative phosphorylation is the principal mechanism of the energy metabolism of normal cells.

It was therefore decided to test the specificity of action of DNP as an uncoupler of oxidative phosphorylation. For this purpose, the action of FCP, another uncoupler of oxidative phosphorylation with a maximal uncoupling concentration two orders of magnitude lower than that of DNP [6], was studied on the same cells. As Table 2 shows, FCP in a concentration of $1 \cdot 10^{-6}$ M, inhibited the deposition of neutral red granules both by L cells and by normal fibroblasts.

DNP and FCP, uncouplers of oxidative phosphorylation, also affected the accumulation of dye by the cells.

Changes in the accumulation of dye by the cells depending on the DNP concentration are shown in Fig. 1. With an increase in the DNP concentration to $1 \cdot 10^{-4}$ M the accumulation of dye both by the L cells and by normal fibroblasts was reduced. A further increase in the uncoupler concentration led to an increase in the amount of dye in the cells because of an intensification of diffuse staining. Accumulation of the dye by the cells was modified in the same way by FCP (Fig. 2).

Normal fibroblasts, unlike L cells and Ehrlich's ascites carcinoma cells [4], thus remain capable of depositing neutral red granules in the presence of $1 \cdot 10^{-4}$ M DNP. Meanwhile, FCP, in the concentration giving maximal uncoupling (1×10^{-6} M), inhibits deposition of neutral red granules both by L cells and by normal mouse fibroblasts. Accumulation of the dye by normal and tumor cells is inhibited by both DNP and FCP. It is difficult to explain these results from the standpoint of the action of uncouplers on the energy metabolism of the cell. In fact, DNP inhibits granule deposition in L cells, the principal mechanism of whose energy metabolism is glycolysis, but has no effect on the deposition of dye granules in normal cells, the energy for which is supplied by oxidative phosphorylation.

The study of the action of several inhibitors of energy metabolism on deposition of neutral red granules in Ehrlich's ascites carcinoma cells by the present writers showed that the cells contain an extramitochon-

drial system, evidently of membrane nature, that is responsible for granule deposition and differs in its sensitivity to external agents from the mitochondrial respiratory chains.

Differences in the action of DNP on the deposition of neutral red granules in normal and L fibroblasts may be connected with differences in the sensitivity of this system to DNP in normal and tumor cells.

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