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Decrease of NADH in HeLa Cells in the Presence of Transferrin or Ferricyanide

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Summary: The short-term incubation of HeLa cells in the presence of diferric transferrin or ferricyanide, which are reduced externally by the transplasma membrane reductase, produces a stoichiometric decrease in NADH and increase in NAD^+, which is stimulated by insulin. The NADP/NADPH ratio does not change during 15 min incubation with the oxidants. The total pyridine nucleotide pool of HeLa cells is not affected. Incubation with apotransferrin and ferrocyanide, which cannot act as oxidants for transmembrane electron transport, does not change the pyridine nucleotide concentrations in the cells. Our results show that NADH can act as the internal electron donor for the reduction of external oxidants by the transmembrane reductase. It appears that oxidation of NADH by the transmembrane electron transport using ferricyanide or iron transferrin as external electron acceptors is sufficient to stimulate growth in HeLa cells. © 1986 Academic Press, Inc.

There are several lines of evidence that a transplasma-membrane redox system is involved in the control of cell growth and development (1). The stimulation of cell growth in serum deficient media by impermeable oxidizing agents (2-5) has been shown. Insulin increases growth of HeLa cells with ferricyanide and increases the rate of ferricyanide reduction by HeLa cells (6,7). Diferric transferrin which is an essential growth factor for many cells can act as an electron acceptor for the plasma membrane redox system (8). Oxidants which are not reduced by the transmembrane electron transport do not stimulate growth (4).

All the plasma membranes which have been prepared show NADH dehydrogenase activities (9,10), but the major problem in the study of the transmembrane redox system in intact cells is to identify the electron donor (1). From indirect evidence, NADH appears to be the endogenous electron donor in erythrocytes (11), HeLa cells (3), and plants (12,13). On the other hand NADPH also may act as an electron donor for iron reduction by some plant cells (14).

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In this report we show that both ferricyanide and transferrin, acting as electron acceptors for the transmembrane redox system stimulate the oxidation of NADH but not NADPH in HeLa cells. Apotransferrin and ferrocyanide which neither stimulate growth (8,4) nor are electron acceptors, do not change the NAD^+/NADH ratio. The decrease of the NADH concentration in response to transferrin and ferricyanide provides direct evidence that NADH is a primary endogenous electron donor for the transmembrane redox system of HeLa cells.

MATERIAL AND METHOD: HeLa cells are grown in an atmosphere 5% CO_2, 95% air on α modified minimal essential media (Gibco) at 37°C with 10% fetal bovine serum, 100 u. penicillin and 100 μg/ml streptomycin at pH7.4. Monolayer cultures are prepared for study by short trypsin treatment to release cells followed by centrifugation at 15,000 xg. Cells were harvested in the same medium without serum during exponential growth. Harvested cells (0.2 g cell wet weight/ml) were treated with different concentrations of oxidant for 5 to 20 min. After the treatments, the pyridine nucleotide pool was determined by extraction of NAD or NADP and NADH or NADPH with perchloric acid and sodium hydroxide, respectively, as described previously (15). The extracted pyridine nucleotide was then quantitated using the cycling assay procedure of Matsumara and Miyachi (16).

RESULTS & DISCUSSION: Pyridine nucleotides were determined in HeLa cells during a short-term incubation with diferric transferrin or ferricyanide with or without 30 μg/ml insulin. Both diferric transferrin (Fig. 1A) and ferricyanide (Fig. 2A) induced an increase in NAD^+, which was proportional to the decrease in NADH. When the incubation was in the presence of insulin, there was an increased effect of the external oxidants on NADH levels. Insulin, a major serum factor known to stimulate cell growth (17), did not itself produce any effect on pyridine nucleotide level in HeLa cells after an incubation for 15 min (data not shown).

In contrast to NADH/NAD^+, NADPH/NADP remained constant. No change in NADPH/NADP was seen either with diferric transferrin- or ferricyanide-treated HeLa cells (Figs. 1B and 2B).

To show that the decrease in NADH and the corresponding increase in NAD^+ depended on the oxidant capacity of the external agent, the NADH contents of HeLa cells were determined after incubation for 15 min with apotransferrin or ferrocyanide. Neither compound caused NADH oxidation nor did they stimulate growth (4,8,17).

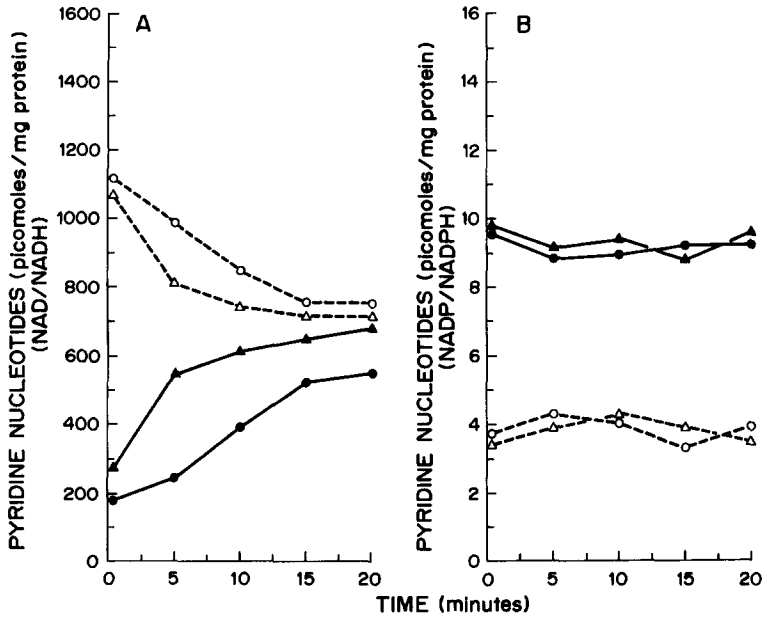

Fig. 1. The short-term effect of diferric transferrin on pyridine nucleotide levels in HeLa cells.

A; ●-●-●, NAD in the presence of diferric transferrin (3.4 μM); 0-0-0, NADH in the presence of diferric transferrin (3.4 μM); ▲-▲-▲, NAD in the presence of diferric transferrin (3.4 μM) and insulin (30 μg/ml); Δ-Δ-Δ, NADH in the presence of diferric transferrin (3.4 μM) and insulin (30 μg/ml).

B; ●-●-● NADP in the presence of diferric transferrin (3.4μM); 0-0-0, NADPH in the presence of diferric transferrin (3.4 μM); ▲-▲-▲, NADP with the presence of diferric transferrin (3.4 μM) and insulin (30 μg/ml); Δ-Δ-Δ NADPH in the presence of diferric transferrin (3.4 μM) and insulin (30 μg/ml)>

Changes in NADH were proportional to diferric transferrin concentration (Table I). Again, both NADPH and total pyridine nucleotide pools were not affected significantly. On the other hand, apotransferrin did not produce any change in the pyridine nucleotide pool. Similar results were obtained comparing ferricyanide with ferrocyanide (Table 2). NADH levels changed in proportion to the amount of ferricyanide added. There was no change in presence of ferrocyanide. Neither NADPH nor total pyridine nucleotide pools were affected by either ferricyanide or ferrocyanide. Iron transferrin and ferricyanide both stimulated growth (4,7,17) and both acted as electron acceptors for the transplasma membrane dehydrogenase (3,18). They also induced proton release across the membrane (3,4,18) which would therefore

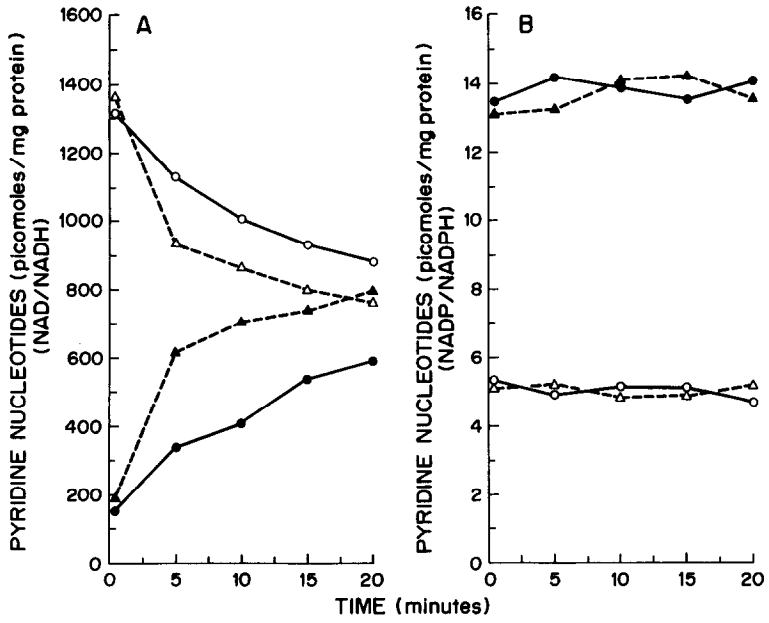

Fig. 2. The short-term effect of ferricyanide on pyridine nucleotide levels in HeLa cells.

A; ●-●-● NAD in the presence of potassium ferricyanide (0.033mM); 0-0-0, NADH in the presence of potassium ferricyanide (0.033mM) and insulin (30 μg/ml). ▲-▲-▲, NAD in the presence of potassium ferricyanide (0.033mM) and insulin (30 μg/ml); Δ-Δ-Δ NADH, in the presence of potassium ferricyanide (0.33 mM) and insulin (30 μg/ml).

B; ●-●-● NADP in the presence of potassium ferricyanide (0.033 mM); 0-0-0, NADPH in the presence of potassium ferricyanide (0.033 mM); ▲-▲-▲, NADP in the presence of potassium ferricyanide and insulin (30 μg/ml); Δ-Δ-Δ NADPH in the presence of potassium ferricyanide (0.033 mM) and insulin (30 μg/ml).

Table 1. Short-term effect of diferrictransferrin and apotransferrin on distribution of pyridine nucleotides of HeLa cells

TREATMENT	PYRIDINE NUCLEOTIDE (picomoles/mg protein)				
	NAD^+	NADH	NADP	NADPH	TOTAL
None	224.	1,367.	8.5	4.4	1,603.
1.7 μM diferrictransferrin	333.	1,247.	8.3	4.4	1,593.
3.4 μM "	553.	1,028.	8.6	3.7	1,693.
1.7 μM apotransferrin	221.	1,369.	9.1	3.0	1,602.
3.4 μM "	225.	1,357.	8.8	3.8	1,595.

Table 2. Short-term effect of ferricyanide and ferrocyanide on the concentration of pyridine nucleotides of HeLa cells

TREATMENT	PYRIDINE NUCLEOTIDE (picomoles/mg protein)				
	NAD^+	NADH	NADP	NADPH	TOTAL
None	126.	1,228.	14.25	5.25	1,373.
0.01 mM ferricyanide	160.	1,182.	13.75	5.10	1,360.
0.01 mM "	424.	917.	15.35	5.15	1,362.
0.01 mM ferrocyanide	134.	1,216.	12.90	5.75	1,368.
0.01 mM "	125.	1,226.	13.40	6.30	1,371.

increase the pH of the cytoplasm. An increased cytoplasmic pH has been associated with cell division in mammalian cells (19-21).

NADH levels have been implicated previously as important to the proliferative state of nontransformed (14,22) as well as SV40 transformed 3T3 cells (14). In rat livers after administration of a carcinogen, 2-acetylaminofluorene, a rapid decline in NADH-requiring enzymes has been reported to follow a marked reduction of NADH pools (23). The latter is due to a greatly enhanced rate of utilization of NADH for the synthesis of poly (ADP ribose) during the excision repair of DNA (23-25). Additionally, it may be reasonable to postulate that the pyridine nucleotide pools are important in the control of cell division. Our results show a relative interchange of the reduced and oxidized forms of NADH induced by external electron acceptors transferrin and ferricyanide, but not of apotransferrin and ferrocyanide. Insulin stimulates the decrease in internal NADH and reduction of the external electron acceptor at concentrations of insulin which stimulates growth of the cells. Thus, not only does NADH serve as a primary endogenous electron donor for the transmembrane redox system of HeLa cells but the stimulation of cell growth by external impermeable oxidants also may be correlated to the oxidation of the internal NADH.

ACKNOWLEDGMENTS

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