EFFECT OF HEMIN AND ISONICOTINIC ACID HYDRAZIDE ON THE UPTAKE of IRON FROM TRANSFERRIN BY ISOLATED RAT LIVER MITOCHONDRIA

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SUMMARY. Isolated rat liver mitochondria accumulate iron from the suspending medium when [^{59}Fe] transferrin is used as a model compound. The accumulation proceeds by two different mechanisms, i.e. by an energy-dependent and an energy-independent mechanism. The energy-dependent uptake of iron from transferrin is inhibited by hemin and stimulated by isonicotinic acid hydrazide. The energy-independent uptake of [^{59}Fe] transferrin is influenced neither by hemin nor by isonicotinic acid hydrazide.

The pattern of iron exchange between transferrin and mammalian cells is very similar in reticulocytes (1), bone marrow cells (2) and hepatocytes (3). The following four steps have been recognized: 1) adsorption of transferrin to the cell surface immediately following mixing of protein and cells, 2) a progressive uptake of further transferrin by the cells during subsequent incubation, 3) a metabolic-dependent release of iron from transferrin to the cells, and 4) release of transferrin from the cells back to the incubation medium

Recently, from experiments with [^{59}Fe,^{125}I] transferrin it has been shown that isolated rat liver mitochondria accumulate iron from transferrin by mechanism(s) essentially as reported for whole cells, i.e. after an initial energy-independent

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Abbreviations used: ALA-synthetase, δ-aminolaevulinic acid synthetase; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethane-sulphonic acid; INH, isonicotinic acid hydrazide; CCCP, carbonyl cyanide m-chlorophenyl-hydrazone; PIPES, piperazine-N,N'-2-bis(2-ethanesulphonic acid).

adsorption of transferrin, there is an energy-dependent release of iron from transferrin, showing a pH optimum, typical saturation kinetics and temperature dependency (4).

The uptake of iron from transferrin by whole cells is inhibited by hemin (5), and markedly stimulated by INH (6). The mechanism(s) by which hemin reduces the uptake of iron from transferrin is unknown. Recently, Ponka and Neuwirt (7), have suggested that hemin might reduce the cellular concentration of cAMP which in turn decreases the rate of iron release from transferrin. The stimulation by INH of the uptake of iron from transferrin has been ascribed to an inhibition of the ALA-synthetase reaction, followed by a reduction of the intracellular concentration of hemin (8,9).

The present paper deals with the effect of hemin and INH on the uptake of iron from transferrin by isolated rat liver mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria were prepared in 0.25 M sucrose, 5 mM HEPES buffer, pH 7.40 at a concentration of approx. 40 mg protein/ml essentially as previously described (4). [^{59}Fe]-transferrin was prepared as described by Martinez-Medellin and Schulman (10).

The concentration of iron was determined by the bathophenanthroline reaction (11). Protein was determined using the Folin-Ciocalteau's reagent (12).

Accumulation of [^{59}Fe] transferrin was determined by incubating the mitochondria containing in a final volume of 6.8 ml: 5 mM succinate, 50 mM glucose, 175 mM sucrose, 5 mM $MgCl_2$, 5 mM KP_i and 10 mM PIPES buffer, pH 7.2. Temperature 30°C. Further additions were as shown in the legends to the figures.

At the time indicated aliqouts were withdrawn, and the mitochondria were rapidly sedimented by centrifugation in an Eppendorf microcentrifuge (Type 3200). The pellet was rinsed with ice-cold 0.25 M sucrose and counted in a well-type scintillator (LKB-Wallac 80000 Gamma Sample Counter) to a precision of ± 2%.

Mitochondrial respiration rates were determined by a Clark oxygen electrode. Mitochondria, approx. 4 mg of protein were suspended in a total volume of 3 ml in the medium of the accumulation experiments (see above). Temperature 30°C. Additions were made as indicated in the legend to the figure.

For the pH experiments, the mitochondria, approx. 9 mg of protein were suspended in a total volume of 6 ml: 0.25 M sucrose containing 2 mM HEPES buffer, pH 7.4. Temperature 30°C. External pH was measured by a semi-micro combination pH electrode (Radiometer, Copenhagen, Denmark) connected to a Radiometer pH meter model PHM 64 with external recorder.

RESULTS.

When freshly prepared mitochondria were incubated with [^{59}Fe]-transferrin and succinate as substrate in the absence and presence of uncoupler (CCCP), marked difference in the accumulation pattern was observed (Fig.1 A and B). Thus, whereas energy-independent accumulation (i.e. the accumulation in the presence of CCCP) increased almost linearly for approx. 60 min, and amounted to 8-12 pmoles iron/mg protein, the energy-dependent accumulation (i.e. the accumulation in the absence of CCCP minus the accumulation in the presence of CCCP) reached a saturation level of 30-40 pmoles/mg protein within 30-40 min. When the mitochondria were incubated with 0.5 mM INH, the energy-dependent accumulation was increased by approx. 100% (Fig.1 A).

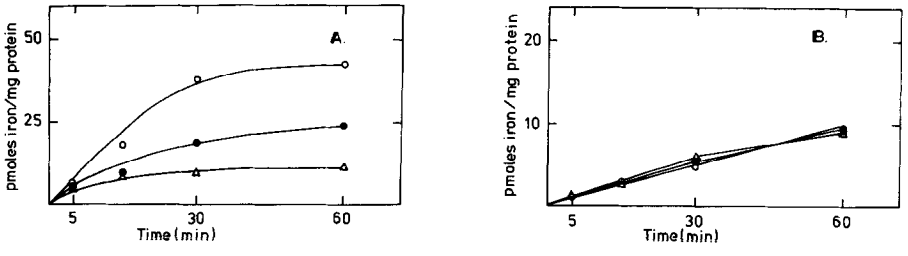

Fig. 1 Time course of the energy-dependent (A) and the energy independent (B) accumulation of [^{59}Fe] transferrin by isolated rat liver mitochondria. Mitochondria at a protein concentration of approx. 2 mg/ml were incubated as described (see Method section) without further addition (●), in the presence of 8 µM hemin (Δ) or 0.5 mM INH (o). The concentration of iron and transferrin in the incubation medium were 2.6 µM and 2.4 µM respectively.

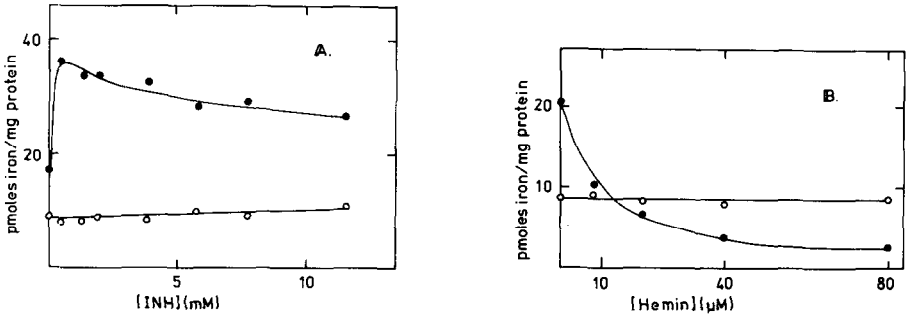

Fig. 2 Effect of increasing concentration of INH (A) and hemin (B) on the energy-dependent (●) and the energy-independent accumulation (o) of [^{59}Fe] transferrin by isolated rat liver mitochondria. Experimental conditions as described in Fig. 1. The reaction was terminated at 30 min.

By increasing the concentration of INH beyond 1 mM, the stimulation was slightly reduced (Fig.2 A). The energy-independent accumulation of iron-transferrin was not influenced by INH (Figs.1 B and 2A).

In the presence of 8 μM hemin, the energy-dependent accumuletion of iron was depressed by approx. 50%, but in contrast to the results with INH, the inhibition increased with increasing concentrations of hemin (Figs. 1 A and 2 B). However, as reported for INH hemin had no effect on the energy-independent accumulation of iron-transferrin (Fig. 2 B).

It has been suggested that hemin may inhibit the transfer of electrons in the respiratory chain, which is essential for the release of iron from transferrin (1,13). As shown in Figs. 3 and 4 neither hemin nor INH had any effect either on the State 3 respiration rate or on the proton permeability of isolated rat liver mitochondria.

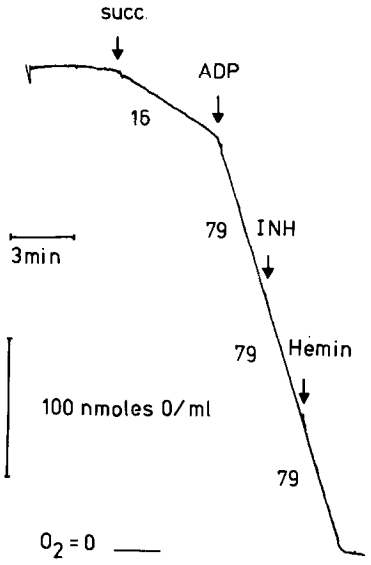

Fig. 3 Effect of INH and hemin on the respiration rate of rat liver mitochondria. The mitochondria were suspended in the standard medium (see Method section). The additions were 5 mM succinate, 0.83 mMADP, 2 mM INH and 50 µM hemin. The numbers below the trace represent the specific respiration rate (natoms oxygen min^{-1}. mg $protein^{-1}$) in the time interval indicated.

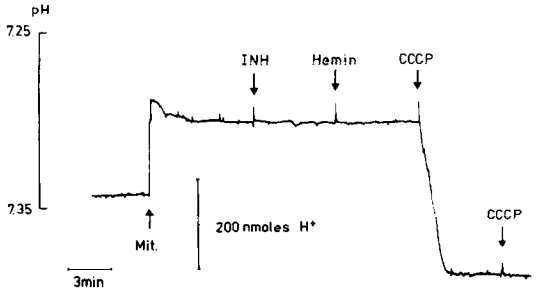

Fig. 4 Effect of INH (2mM) and hemin (50 µM) and CCCP (3 µM) on the external pH in suspended rat liver mitochondria. Experimental conditions as described (see Method section).

DISCUSSION.

In intact cells, hemin has been reported to interfere with the synthesis of heme 1) by acting as a repressor of the ALA-synthetase at the post-transcriptional level (14) and 2) by

inhibiting the metabolic dependent release of iron from transferrin (7,9,15).

As shown in the present study, hemin at concentrations similar to those necessary to depress the release of iron from transferrin by erythroid cells (15,16), inhibits the energy-dependent release of iron from transferrin by isolated rat liver mitochondria as well. Thus, our results fit the suggestion of Neuwirt et al. (17) that hemin may function as a feedback regulator of the release of iron from transferrin at the inner mitochondrial membrane. This conclusion is further supported by the recent finding that the ferrochelatase reaction of intact mitochondria, utilizing transferrin and deuteroporphyrin as substrates, is markedly inhibited by hemin, whereas in sonicated mitochondria hemin has only a negligible effect on the ferrochelatase reaction (M.Koller, manuscript under preparation). A regulatory function of the intracellular heme-pool on the mitochondrial accumulation of iron has been suggested also from findings in sideroblastic anemias (13,18,19). Thus, the 'ring' of sideroblasts which consists of iron-loaded mitochondria is probably the results of an absolute increase of iron entry into the erythroblasts secondary to a primary inhibition of heme synthesis, and a decreased utilization of iron (13,18,19).

The mechanism(s) by which hemin controls the release of iron from transferrin is largely unknown. From studies in intact cells it has been suggested that hemin inhibits the flow of electrons in the respiratory chain (13) which is of importance to the metabolic dependent release of iron from transferrin (1,20). In isolated mitochondria the effect of hemin could not be explained by this mechanism. Thus, in the present experi-

ments, hemin at concentrations 5 times those necessary to depress the energy-dependent accumulation of iron by 50% has no effect either on the State 3 respiration rate or on the proton permeability of the inner membrane (Figs. 3 and 4). Furthermore, our results could hardly be explained by a reduction of the ALA-synthetase activity, because under conditions of inhibited ALA-synthetase activity there is usually a large iron loading of the mitochondria (18).

Concerning the effect of INH, our results are essentially as those reported by Borova et al. (9) from experiments with rabbit reticulocytes. This is to be expected from the localization of ALA-synthetase in the mitochondrial matrix (21), the inhibition by INH of ALA-synthetase (8), and the subsequent reduction of the heme pool (9).

Thus, our results indicate that rat liver mitochondria possess a metabolic dependent (4), hemin-sensitive mechanism (Figs.1 and 2) capable of releasing iron from transferrin. Although recent experiments indicate that transferrin may enter the cells possibly by endocytosis (22,23), so far, we do not know with certainty whether or not transferrin functions as a donor of iron to mitochondria in situ (24). Work is now in progress in this laboratory aimed at studying the possible role of transferrin in the intracellular iron metabolism.

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