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THE REGULATION OF LIVER IRON METABOLISM

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Abstract

^{55}Fe-transferrin and ^{59}Fe-ferritin were injected simultaneously in rats and storage iron release by the hepatocyte was calculated from the cumulative incorporation of the two isotopes in the red cell mass over 2 weeks. About 6 % of hepatocyte storage iron was released daily in normal rats, but a non-mobilizable pool was identified in iron overload. Iron release was regulated by the rate of erythropoiesis and iron status of the animal, while acute inflammation blocked iron release only transiently.

Introduction

Hepatocyte iron release to the plasma is less well characterized than iron uptake by the liver (1). Two phases of iron release can be distinguished, i.e. (a) an immediate release during the initial processing of transferrin, hemoglobin, ferritin, or "free" iron (early release, ER), and (b) iron release from stores (late release, LR). We present the results of studies of storage iron release in normal rats and animals with various disorders of iron status and erythropoiesis, using ^{59}Fe-labelled ferritin as a selective tag of hepatocytes (2,3,4).

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Methods

Rat transferrin was purified by saturating plasma transferrin with ferrous sulfate, followed by ammonium sulfate precipitation, ion-exchange chromatography using a DEAE Sephacel column and gel chromatography using a S-200 Sephacryl column. Apotransferrin was prepared by incubating diferric transferrin with desferrioxamine, which was removed afterwards by ultrafiltration. Labeling of apotransferrin with ^{55}Fe ferrous sulfate was achieved in the presence of 0.05 M Na bicarbonate and 0.1 M Tris/HCl. The transferrin solution was brought to the point of saturation by adding cold ferrous sulfate. Ferritin was purified from rat liver by heating at 75°C for 10 minutes, ammonium sulfate precipitation, gel chromatography using a Sepharose 6B column, and ultracentrifugation (3). In vitro tagging of ferritin was achieved by adding $^{59}FeSO_4$ to a normal rat ferritin preparation in 0.1 M Hepes buffer (pH 7.5). The labelled ferritin was purified by ion-exchange chromatography using a DEAE Sephacel column (3). Male Sprague-Dawley rats 8 to 10 weeks of age were used in most experiments. Animals to be made iron deficient were placed at 4 weeks on a diet containing 4-8 mg of iron/Kg, or alternatively were given an iron deficient diet at 6 weeks of age and bled 2-3 ml 4 to 5 times over two weeks to accelerate iron depletion. Female rats to be iron-loaded were placed on a 1% carbonyl iron diet for > 1 year with occasional reintroduction of a normal diet to maintain animals in good health. In order to suppress erythropoiesis, rats were transfused with 3 ml packed red cells approximately twice a week to maintain an hematocrit of 60-65 %, and ^{59}Fe ferritin was injected on the 5th day after the first transfusion. Hemolytic anemia was produced by the intraperitoneal injection of 50 mg acetylphenylhydrazine per Kg body weight, and ^{59}Fe ferritin was injected 5 days later. Inflammation was produced by injecting 0.25 ml turpentine oil in each thigh, and ^{59}Fe ferritin was injected 16 hours later.
Plasma was drawn from a rat prepared in the same way as the experimental animals and labelled by adding 0.5 µCi ^{55}Fe as $FeSO_4$ in 0.9% saline (pH 2) to 0.5 ml plasma. The rate of radioiron disappearance (t-1/2) was determined and the plasma iron turnover (PIT) was calculated in µg/Kg/day:

$$PIT = \frac{SeFe\ (\mu g/dl) \times (100 - Hct \times 0.92) \times 7}{t\text{-}1/2\ (min)}$$

Release of storage iron by the hepatocyte (i.e. late release) was studied over a period of 14 days. The cumulative incorporation of ^{59}Fe (injected as ferritin) and ^{55}Fe (injected as diferric transferrin) in the red cell mass was determined over a 2 wk period. The ratio of ^{59}Fe to ^{55}Fe activity in the red cell mass at any time represents the total fraction of ^{59}Fe released by the hepatocyte (total release at time t = early release + late release at time t). When the complement of this ratio was plotted against time on a semilogarithmic paper, late release appeared to be a single exponential curve. The proportion of the injected dose released in the early and late phases was obtained by extrapolating the curve to zero time. The t-1/2 of the curve, determined by least squares, represents the speed of late release and was expressed as % of the dose/day. Formal proof of this method has been published (5). Labelled compounds were injected into a tail vein and blood samples were obtained from the tail vein on the opposite side. At the end of each experiment, rats were exsanguinated from the abdominal aorta, and perfused with 20-30 ml saline. Whole blood, plasma, and liver activities were counted. Total plasma and total red cell activities were calculated from the hematocrit and assuming a blood volume of 0.07 ml/g body weight. Non-heme iron was extracted from rat livers and iron was determined by Proton-Induced X-ray Emission (PIXE). Assuming that ferritin ^{59}Fe uniformly labelled non-heme iron stores in the hepatocyte, the amount of iron released daily by the hepatic parenchyma was calculated as the product of radioiron release (%/day) and hepatocyte iron stores (μg).

Results

Measurements of the PIT and late release are presented in table 1.

Table 1. Plasma iron turnover and storage iron release.

Group	PIT (μg/Kg/d)	LR (%)	LR (%/day)	LR (μg/Kg/d)
Normal males	1815±381	78±8	6.3±0.9	207±48
Normal females	1612±202	91±2	3.7±0.3	347±32
Iron deficiency	738±3	38±3	17.0±4.6	79±26
Iron overload	1395±316	96±1	1.6±0.5	1584±650
Phenylhydrazine	6802±1961	56±14	14.9±6.2	331±75
Hypertransfusion	654±222	96±1	0.9±0.1	81±3
Inflammation	1322±60	93±2	6.4±2.7	193±96

Normal males released 17.5% of the ferritin ^{59}Fe in the early phase. Thereafter, 6.3% of the residual hepatocyte radioiron was released daily, representing a total of 207 μg Fe/Kg/day, i.e. about 11% of the PIT. Normal females had a slower rate of late release. However, as their iron stores were consistently higher, the contribution of hepatocyte iron to the PIT was found to be higher than in males (21%). Iron turnover in the hepatocyte of iron deficient rats is extremely rapid (56% ER and thereafter 17%/day of LR) but involves relatively small amounts of iron, a little more than 10% of the PIT. By contrast, iron overloaded rats had a turnover about 10 times slower (6% ER and 1.6%/day of LR), amounting to a calculated value of 1584 μg/kg/day, which is an overestimation. Rats treated with phenylhydrazine had an increased amount and rate of iron turnover in the hepatocyte. However, the participation of the parenchymal cell in the PIT was rather small (5%). On the other hand, hypertransfused rats had a slow turnover involving small amounts of iron which represented 12% of the PIT. Inflammation reduced considerably the early release of radioiron. However, release of hepatocyte iron in the following days was normal. There was a very significant correlation between the percent ER and the rate of LR ($R=0.90$, $P<0.001$). Iron release was inversely related to the hematocrit and positively to the PIT. Plasma iron and radioiron t-1/2, as well as hepatic iron, correlated with the rate but not the absolute amount of iron release.

Discussion

After uptake by the hepatocyte, ferritin is catabolized and its iron enters an intermediate pool before being incorporated into stores (3,4). Because it is based on the assumptions that ferritin ^{59}Fe labels all hepatocyte iron stores uniformly and that all non-heme iron is mobilizable in the same way, the calculation of the amount of hepatocyte iron released daily to the plasma is less precise than the measurement of radioiron release. The rate of radioiron release was considerably increased in iron deficient rats but 6.2% of the ferritin ^{59}Fe remained in the liver after 2 wks, indicating that not all hepatic iron is mobilizable by increased marrow requirements. Another pool of non-exchangeable iron, presumably hemosiderin, was identified in iron overloaded rats in which the calculation of iron released by the liver was grossly overestimated. In iron overload, hemosiderin iron represents a large proportion of hepatic iron, which is not exchangeable at least over the time and in the conditions of the present study. Nevertheless, the values obtained in non-deficient and non-overloaded rats are reasonable. Iron release in normal animals amounted to 207 µg Fe/Kg/day, which represents 11.4% of the PIT. These findings are similar to values reported by others (4). This is also in accordance with hepatocyte iron uptake, as approximately 10% of transferrin radioiron is localized in the liver after the early release phase. With iron deficient and iron overloaded rats not included in the analysis, there was a good correlation between hepatic storage iron and the rate of LR, but not with the absolute amount of iron released daily. This observation confirms that ^{59}Fe and cold iron are released in a similar fashion. Inflammation produced a significant decrease in the rate of early radioiron release. However, late release was not affected. These observations confirm a previous study in which the effect of turpentine-induced inflammation on storage iron mobilization was also transient and was no longer apparent beyond 48 hrs (6). However, human studies have shown that chronic inflammation is associated with a blockade of iron release from RE stores (7). The effect of erythropoiesis on

hepatocyte iron mobilization was examined in phenylhydrazine-injected and hypertransfused rats. Iron release was considerably modified in parallel to changes in erythropoiesis. The amount of iron released by the hepatic parenchyma seemed appropriate (12% of the PIT) in hypertransfused rats. However, it accounted for only 4.9% of the PIT in phenylhydrazine-injected animals, indicating that recycling of red cell iron through the reticuloendothelial system contributed more than hepatocyte iron mobilization to increased marrow iron requirements.

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NEW DATA ON THE HYPOTHESIS OF THE BRAIN PARTICIPATION IN IRON HOMEOSTASIS

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Abstract

Ferropenic posthemorrhagic rats were used when hematocrite reached 30% and hemoglobin was lower than 8g/100ml. The brain is homogenized (1:4,w:v), sonicated, centrifuged and the supernatant, is incubated with 2μCi ^{59}Fe for a period of 20h. The subsequent elution of two peaks, supposedly ferritin and metallothionein marked with ^{59}Fe justifies the hypothesis before exposed of the brain participation in iron homeostasis.

Introduction

Metallothionein, a well known low relative molecular weight protein, with approximately 6000 D, 30% cystein and without aromatic aminoacid residues, associates between 5 to 11 atoms of metals (1,2). Its signification in metabolism is not well understood, it is suggested that it could participate as regulatory protein (3). Some data are published before, comparing the increase of transferrin and metallothionein in the intestinal mucosa of ferropenic rats in the brain (5,6).
The object of this work is to furnish new data which justify the hypothesis of the brain participation in iron homeostasis (7,8), exposed after the experimental results on the partici-

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