Effects of Chronic Iron Deficiency Anemia on Brain Metabolism


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Abstract The effects of chronic iron deficiency anemia on brain (cortex) metabolism were estimated by 31P-nuclear magnetic resonance spectroscopy and biochemical analyses in male Wistar rats. Iron deficiency anemia was induced by supplying diet containing either ~2 or ~6 ppm Fe. Control diet was supplemented with 100 ppm Fe as ferric citrate. After 8–9 weeks, blood hemoglobin levels were approximately 13, 5, and 3 g/100 ml in the 100 ppm, 6 ppm, and 2 ppm Fe group, respectively. The blood lactate levels at rest in these groups were approximately 3, 5, and 6 mM. The blood glucose concentration also tended to be elevated in iron-deficient rats. The high-energy phosphate contents in brain were not affected by iron deficiency. The activities of succinate dehydrogenase and cytochrome oxidase per unit protein in the 2 ppm Fe group were significantly less than in the 100 ppm Fe group, but those activities were not significantly affected by feeding diet with 6 ppm Fe. The activities of lactate dehydrogenase in iron-deficient group tended to be elevated but not significantly. The activities of non-iron containing mitochondrial enzymes, citrate synthase and β-hydroxyacyl CoA dehydrogenase, were unchanged. It is suggested that the brain has a higher tolerance to iron deficiency than skeletal muscle in terms of the metabolic characteristics, although this may be associated with a lower level of neural activity.

Key words: iron deficiency anemia, brain metabolism, phosphorus compounds, enzymes.

Decreased physical work performance is one of the pronounced characteristics

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caused by iron deficiency anemia both in humans and animals [1–4]. This phenomenon is closely related to the decreased oxygen-carrying capacity of blood and oxygen utilization in muscles, since iron deficiency inhibits synthesis of both blood hemoglobin (Hb) and iron-containing protein in tissues [5–9]. A reduced voluntary activity [10, 11] and altered mental function such as behavior patterns and/or learning capability [12, 13], which may be affected by brain metabolism, are also caused by iron deficiency anemia. These observations suggest that brain function may also be influenced by either iron deficiency, anemia, or both.

Mackler et al. reported that the respiratory capacity and formation of adenosine triphosphate (ATP) in brain were normal in iron-deficient and anemic rats with Hb below 7 g/100 ml, although abnormal serotonin metabolism [14] and increased blood levels of phenylalanine [15] which may affect the brain function were observed. However, it is not known how the brain metabolism is affected if the magnitude of iron deficiency and anemia is progressed further.

The activities of non-iron-dependent mitochondrial enzymes are elevated if iron deficiency is severe enough to reduce the high-energy phosphate contents in skeletal muscle of rats with mean blood Hb of 3.5 g/100 ml [9]. However, the responses of brain metabolism in these animals are not known. Therefore, the current study was carried out to investigate how the energy metabolism in the brain respond to severe iron deficiency anemia in rats.

MATERIALS AND METHODS

Twenty-four newly weaned male Wistar rats with mean ± SEM body weight of 54.5 ± 3.7 g were randomly separated into normal control and two iron-deficient groups (n = 8 in each group). They were pair-fed either powdered iron-deficient diet with ~2 ppm (170365, Teklad, Madison, WI, USA) or ~6 ppm Fe (TD 77349, Teklad) or control diet with 100 ppm Fe (ferric citrate) supplementation (TD 92118, Teklad) for 8–9 weeks. All of the food was eaten within approximately 12 h. The daily food supply was gradually increased, but 20 g of diet was given to each rat after the 4th week. Deionized water was supplied ad libitum. Rats were housed individually in stainless steel cages. The temperature and relative humidity in the animal room with a 12-h light–dark cycle were maintained at approximately 23°C and 55% throughout the study.

After 8–9 weeks, the distributions of ATP, phosphocreatine (PCR), and inorganic phosphate (Pi) in the brain of rats anesthetized by intraperitoneal injection of sodium pentobarbital (5 mg/100 g body weight) were analyzed by using 31P-nuclear magnetic resonance (NMR) spectroscopy (Ohtsuka Electronics, BEM 170/200, Tokyo). The rat was placed on a probe table in a prone position after exposing the skull. A 2-turn surface coil with 20-mm diameter was placed above the skull and whole body was put into a 170-mm-bore-diameter superconducting magnet (4.7 T). The analysis was performed at a resonance frequency of 81.4 MHz, a repetition time of 3.0 s, and a radio frequency pulse width of 26 μs. The
free induction decay signal was accumulated for 150 times. The $P_i$/PCr, PCr/(PCr + $P_i$), and PCr/ATP ratios were calculated by using the peak heights of $P_i$ and PCr.

Blood was withdrawn from the jugular vein into a heparin-coated syringe for measurement of hematocrit (Hct), Hb (Autoanalyzer, Nihon Technicon, MEK-4500), and lactate (Lactate Analyzer, YSI, Model-23L). The rat was then killed with excess sodium pentobarbital. The brain cortex was removed and weighed. The tissues were homogenized in 0.3 M sucrose buffer with 10 mM Tris and 2 mM ethylenediaminetetraacetic acid (pH 7.2) at 0°C using a polytron. The test tubes containing homogenized samples were kept in ice until spectrophotometrical analyses of the activities of cytochrome oxidase (CO) [16, 17], succinate dehydrogenase (SDH) [18], and lactate dehydrogenase (LDH) [19] in the same day. $\beta$-Hydroxyacyl CoA dehydrogenase (HOAD) [20] and citrate synthase (CS) [21] activities were measured after the samples were frozen at $-80^{\circ}$C and thawed at least three times. The protein concentration was measured by the method of Lowry et al. [22]. Statistical analyses were performed using analysis of variance and Student's $t$-test.

RESULTS

Significantly lower body weights were noted in rats fed iron-deficient diets (Table 1, $p < 0.05$), but the body weight was not affected by the severity of iron deficiency (6 vs. 2 ppm Fe diet). The absolute brain weight was not affected by feeding iron-deficient diet, and this may be due to the fact that the brain development is rapid and the growth is almost completed at the weaning stage [23, 24] when the feeding of iron-deficient diets was initiated. Severe iron deficiency anemia with Hb and Hct levels of $5.2\pm0.2$ (mean±SEM) g/100 ml and $15.0\pm2.3\%$ was induced by feeding diets containing approximately 6 ppm Fe (Table 1, $p < 0.001$). The Hb levels were reduced further by feeding the 2 ppm Fe diet ($p < 0.05$). Anemia was associated with an elevation of resting blood lactate concentration. The lactate level tended to be greater in the 2 ppm Fe group than 6 ppm Fe group but the difference was not significant. Further, blood glucose concentration of the iron-deficient rats tended to be greater than in the control rats. That in the 6 ppm

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Hematocrit (%)</th>
<th>Lactate (mM)</th>
<th>Glucose (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm Fe</td>
<td>$215\pm8$</td>
<td>$12.8\pm0.8$</td>
<td>$40.9\pm1.6$</td>
<td>$2.29\pm0.43$</td>
</tr>
<tr>
<td>6 ppm Fe</td>
<td>$187\pm8^{*}$</td>
<td>$5.2\pm0.2^{***}$</td>
<td>$15.0\pm2.3^{***}$</td>
<td>$5.12\pm0.51^{**}$</td>
</tr>
<tr>
<td>2 ppm Fe</td>
<td>$183\pm9^{*}$</td>
<td>$3.4\pm0.1^{***, \dagger}$</td>
<td>$14.1\pm1.3^{***}$</td>
<td>$6.00\pm0.33^{***}$</td>
</tr>
</tbody>
</table>

Mean±SEM. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. 100 ppm Fe (control) group, and $^{\dagger}p < 0.05$ vs. 6 ppm Fe group.
Table 2. Effects of iron deficiency on the distribution of high-energy phosphates in brain.

<table>
<thead>
<tr>
<th></th>
<th>P$_i$/PCr</th>
<th>PCr/(PCr + P$_i$)</th>
<th>PCr/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm Fe</td>
<td>0.33±0.31</td>
<td>0.75±0.02</td>
<td>0.94±0.10</td>
</tr>
<tr>
<td>6 ppm Fe</td>
<td>0.36±0.04</td>
<td>0.74±0.02</td>
<td>0.85±0.12</td>
</tr>
<tr>
<td>2 ppm Fe</td>
<td>0.32±0.04</td>
<td>0.76±0.02</td>
<td>0.98±0.08</td>
</tr>
</tbody>
</table>

Mean±SEM. P$_i$: inorganic phosphate, PCr: phosphocreatine, ATP: adenosine triphosphate.

Table 3. Effects of iron deficiency anemia on enzyme activities (μmol/min/g protein) in brain.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>100 ppm Fe</th>
<th>6 ppm Fe</th>
<th>2 ppm Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase</td>
<td>105±12</td>
<td>100±18</td>
<td>74±7*</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>114±12</td>
<td>97±10</td>
<td>80±10*</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>247±34</td>
<td>229±30</td>
<td>226±14</td>
</tr>
<tr>
<td>$\beta$-Hydroxyacyl CoA dehydrogenase</td>
<td>48.5±2.8</td>
<td>45.8±7.9</td>
<td>38.8±2.8</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1,164±157</td>
<td>1,206±125</td>
<td>1,313±110</td>
</tr>
</tbody>
</table>

Mean±SEM. *p < 0.05 vs. 100 ppm Fe group.

Fe group was significantly higher than in the 100 ppm Fe group (p < 0.001).

The distribution of high-energy phosphates in the brain estimated by $^{31}$P-NMR was unchanged by feeding iron-deficient diets (Table 2). No statistically significant differences were observed in the P$_i$/PCr, PCr/(PCr + P$_i$), and PCr/ATP ratios between each group. Enzyme activities in brain are shown in Table 3. The activities of iron-containing enzymes, SDH and CO, per gram of protein in 2 ppm Fe group were significantly less than control (p < 0.05), but those activities were not significantly affected by feeding diet with 6 ppm Fe. The activities of non-iron-dependent mitochondrial enzymes, CS and HOAD, were unchanged by feeding iron-deficient diet. The activities of LDH in the iron-deficient groups tended to be elevated but not significantly.

**DISCUSSION**

It has been well reported that severe iron deficiency anemia causes a decrease...
in the level of voluntary activity [10, 11], maximal oxygen consumption [4], and/or endurance work capacity [2-4]. Further, Pollitt et al. [25] reported that children with a Hb ≤ 10.5 g/100 ml had poor scores in learning test than those with a Hb ≥ 11.5 g/100 ml. In another study, abnormal behavior was seen in iron-deficient anemic infants [26]. Anemic infants tended to be less active and persistent and were less responsive, less reactive, more tense, and more fearful than non-anemic infants.

Decreased specific activity of aldehyde oxidase, which is involved in degradation of serotonin, was seen in brain mitochondria of iron-deficient rats [14]. It is also reported that plasma phenylalanine concentration of iron-deficient rats with mean Hb of approximately 6 g/100 ml was higher than normal due to inhibition of conversion to tyrosine, because of the decrease in the activity of iron-containing enzyme, phenylalanine hydroxylase, in the liver [15]. Toxic by-products due to phenylalanine accumulation may lead to an impairment of mental function. These phenomena may affect the behavior and/or learning capability of iron-deficient rats.

However, the cytochrome content of brain mitochondria and their phosphorylation capacity as measured by P:O ratios, rates of ATP formation, and respiratory control indexes were unaffected in iron-deficient anemic rats with a Hb concentration below 7 g/100 ml [14]. Further, we found no significant deficiency of iron in the brain of rats fed an iron-deficient diet and with a mean Hb of 6 g/100 ml [27, 28].

More severe anemia with mean Hb of 5 g/100 ml was induced by feeding iron-deficient diet containing ~6 ppm Fe in our study. However, the distribution of high-energy phosphates in brain was not affected as in skeletal muscle [5]. Even though iron-containing enzyme activities in skeletal muscles were below normal [5], both SDH and CO activities in the brain were not inhibited in the current study.

When the degree of anemia progresses further, the PCr levels in the soleus muscles of resting rats fed 2 ppm Fe diet decreased [9]. In these muscles, non-iron-dependent enzymes, such as CS and HOAD, are activated. However, such phenomena were not seen in the brain. Although the specific activities, per gram of protein, of SDH and CO in the brain were significantly less than those in control rats, none of the high-energy phosphates and non-iron-dependent enzymes were affected. Such phenomena may be associated with a lowered level of neural activity in the brain which may influence the voluntary activity [10, 11], learning capability [25], or behavior [26].

Brain growth may not be affected by the diet, because it is almost completed at weanling [23, 29] when the present experiment was initiated. It is also reported that the turnover rate of iron compounds in the brain is slow [24]. Further, in the brain metabolism, the elevated blood glucose level may compensate for the reduced availability of O₂. These compensatory changes could be other possible factors which maintain the brain metabolism near normal even in rats with muscle metabolism severely affected by iron deficiency. In general, the brain has a higher
tolerance to iron deficiency than skeletal muscles in terms of the metabolic characteristics, although this may be associated with the lower level of neural activity in the brain, demonstrated by reduced voluntary activity [10, 11] and defects in learning capability and behavior [25, 26].

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REFERENCES


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