Mucosal surface ferrocyanide reductase in mouse duodenum

DAVID J. POUNTNEY and ROBERT J. SIMPSON.

Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, Besseney Road, London SE5 9PJ.

The precise mechanism of uptake of iron and its regulation is not fully understood. The absorption process includes at least two distinct steps: (i) uptake of iron across the brush border membrane of the duodenum, and (ii) transfer to the portal circulation. It has been proposed that mucosal surface Fe(III) reduction by a transplasma-membrane redox system precedes and facilitates the uptake of iron from FeNTA₂ (1). Transplasma membrane ferrocyanide reductases have been reported in a variety of tissues and, indeed, an iron-reductase is known to operate in the yeast Saccharomyces Cerevisae and plays a central role in iron assimilation (2). We have further reported in a variety of tissues and, indeed, an iron-reductase is known to operate in the yeast Saccharomyces Cerevisae and plays a central role in iron assimilation (2). We have further shown that intestinal uptake of Fe(III) from FeNTA₂ is inhibited by ferrocyanide (1), possibly by preferentially accepting electrons from a reductase. We therefore set out to determine whether mouse duodenum possesses a transplasma membrane ferrocyanide reductase system which is regulated in parallel with iron absorption.

In this study we have measured ferrocyanide reduction by intact CD-1 mouse intestinal fragments incubated in vitro by two methods: 

A) Ferrocyanide production was determined using the method of Avron and Shavitt (3). This involved incubating fragments with ferrocyanide (1mM), sampling the medium and assaying for ferrocyanide.

B) Ferrocyanide (200µM) disappearance in the presence of tissue fragments was followed spectrophotometrically at 410nm. Scattering due to tissue degradation was corrected for by simultaneous monitoring at 550nm.

Mice with raised levels of iron absorption were obtained by placing them in a hypobaric chamber set at 0.5 atmospheres for 3 days. Iron deficiency was induced by feeding mice on iron deficient diet for three weeks from weaning. Iron replete controls were fed the same diet supplemented with 125mg/kg FeCl₃.

The reducing activity (pmol/mg gut/15 min incubation) by method A was found to be greater in duodenal fragments (161±18) when compared to ileal fragments (836±159) (p<0.05). Duodenal fragments from hypoxic mice had an increased reducing activity (1614±167) (p<0.05), but no hypoxic response was seen with hypoxic ileal fragments (1055±118). Hypoxic mice have been shown to have elevated levels of iron uptake and the parallel increase in reducing activity seen with hypoxic duodenal fragments indicates that reduction and uptake are related steps. The results also suggest that any regulation of the reducing enzyme to adapt to hypoxic conditions is confined to the duodenum. The method for determining ferrocyanide production is not very specific, since when ferrocyanide was omitted from the medium a basal production of reducing activity was observed. This basal activity was however, independent of intestinal location or hypoxia.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Ferrocyanide reduction rate constant (pmol/mg gut/15 min)</th>
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<tbody>
<tr>
<td>normal</td>
<td>0.97±0.11 (5)</td>
</tr>
<tr>
<td>hypoxic</td>
<td>1.84±0.26 (6)*</td>
</tr>
<tr>
<td>Fe replete</td>
<td>1.02±0.24 (6)*</td>
</tr>
<tr>
<td>Fe deficient</td>
<td>2.33±0.56 (6)*</td>
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Table 1 shows the results of determinations of ferrocyanide reduction by the disappearance method, in proximal intestinal tissue from normal, 3 day hypoxic, iron deficient and iron replete mice. Ferrocyanide reduction is increased in both groups of mice which have raised iron absorption (hypoxic, iron deficient). Corresponding values were observed from identical experiments using mucosal (everted) tied-off duodenal sacs from hypoxic and control mice.

Incubation media that had been pre-incubated with duodenal tissue from hypoxic mice for 5 minutes had virtually no capacity to reduce ferrocyanide after removal of the tissue and subsequent addition of ferrocyanide, thus providing evidence against a significant intestinal release of reducing factors. Serosal (non-everted) tied-off duodenal sacs were unable to reduce ferrocyanide; suggesting that the reducing activity is on the mucosal surface.

Both methods of assay (A and B) were examined for their comparability by using the same incubations for each assay (200µM ferrocyanide was used). A good quantitative agreement between the two methods was obtained, using either hypoxic or normal duodenum. Ferrocyanide production for normal duodenal fragments was 3.3±0.4 nmol/mg tissue/9min (n=7) incubation, and 5.2±0.6 (n=6) for hypoxic duodenal fragments. The corresponding results for ferrocyanide disappearance were 3.8±0.9 and 5.3±0.6 respectively. This quantitative agreement suggests that both methods are valid for the determination of ferrocyanide reduction.

The results presented here demonstrate that mouse duodenum, incubated in vitro, is able to reduce ferrocyanide to ferrocyanide. Ferrocyanide does not enter cells, and together with ferrocyanide, is relatively biologically inert. Several cells and tissues have been demonstrated to reduce ferrocyanide using a transplasma membrane reductase (4). This data demonstrates that mouse duodenum possesses a transplasma membrane ferrocyanide reductase which is induced in parallel with increased iron absorption and is localised to the duodenum. The mouse duodenum has recently been shown to possess the capacity to reduce several different iron complexes (FeNTA₂, maltol and citrate) in a similar way. Preliminary results show that ferrocyanide reduction by duodenal fragments from mice with a genetic defect in transferrin regulation is greatly enhanced when compared to that of normal controls. Collectively this provides further evidence that a transplasma membrane ferri-reductase in mouse duodenal mucosa is involved in the intestinal absorption of Fe(III).

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