BBA 75821

IRON INFLUX ACROSS THE BRUSH BORDER OF RABBIT DUODENUM: EFFECTS OF ANEMIA AND IRON LOADING

LOUISE S. ACHESON AND STANLEY G. SCHULTZ

Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, Pa. 15213 (U.S.A.)

(Received August 16th, 1971)

SUMMARY

1. Unidirectional influx of iron across the mucosal border of duodenum was determined in normal, anemic, and iron-loaded anemic rabbits.

2. Unidirectional influx of iron is significantly greater in anemic rabbits than in controls.

3. Unidirectional influx of iron in anemic rabbits treated with intramuscular iron does not differ significantly from control.

4. These results strongly suggest that the stimulation of intestinal iron absorption by iron deficiency involves the mechanism(s) responsible for entry across the mucosal border and cannot be attributed to an effect on the serosal transfer mechanism alone.

INTRODUCTION

It is well established that iron absorption by the small intestine is stimulated in conditions of iron deficiency and depressed by iron loading[1]. At the cellular level, an increased net movement of iron from the lumen across the brush border can result from: (a) a decreased unidirectional efflux of iron from the cell across the mucosal border; (b) an increased unidirectional influx into the cell from the lumen; or (c) a combination of these changes. Although an increase in influx would strongly suggest a primary effect on brush border transport mechanisms, a decreased efflux could be secondary to a decline in intracellular iron resulting from the deficiency state and/or enhanced activity of a transport mechanism located at the serosal membrane.

The purpose of these experiments was to observe the effect of hemorrhagic anemia and iron loading on the unidirectional influx of iron across the mucosal border of rabbit duodenum.

METHODS

Male white rabbits (2–4 kg) which had been maintained on a normal diet for 2–4 weeks were used for all experiments. Immediately before each experiment,

blood for a hematocrit was drawn from the marginal ear vein into a heparinized capillary tube. The rabbit was then killed by intravenous injection of pentobarbital. A section of small intestine immediately distal to the pylorus, approx. 25 cm long, was excised, opened along the mesenteric border, washed free of its contents and mounted in plastic chambers. These chambers are constructed so that eight well-defined areas (each 1.13 cm^2) of the mucosal surface alone can be exposed to bathing solutions which are stirred by bubbling with O_2. Details of the apparatus and procedure have been published previously[2]. The tissue was first preincubated at 37° for approx. 20 min in a solution containing: 140 mM choline chloride, 0.1 mM $CaCl_2$, 0.8 mM ascorbic acid, and 10 mM Tris buffer, pH 7.3. Similar results were obtained when 140 mM NaCl was employed in place of 140 mM choline chloride. Each area of tissue was then exposed for 2 min to an unlabeled solution containing: 140 mM NaCl, 0.1 mM $CaCl_2$, 10 mM Tris, 0.8 mM ascorbic acid, and 1.0 mM $FeCl_2$. Finally, the tissue was exposed to 1 ml of the same solution containing, in addition, ^{59}Fe (International Chemical and Nuclear Corporation) and [^{3}H]inulin (New England Nuclear Corporation) for a predetermined period of time. This test solution was removed and the tissue was quickly rinsed with ice-cold unlabeled test solution, punched out and extracted in 0.1 M HNO_3 containing 50 mM $FeCl_2$ for 3 h. All of the ^{59}Fe is recovered from the tissue within 1–2 h by this procedure. Appropriate samples of the tissue extracts and test media were assayed in a liquid scintillation counter. ^{59}Fe uptake across the brush border was calculated after correction for the tracer content of the inulin space.

In several instances, serum iron concentrations and iron-binding capacities were determined on blood drawn immediately prior to killing using standard procedures[3,4].

Anemic rabbits

Some rabbits were made anemic by drawing 40–50 ml of blood from the central arteries of their ears. Subsequently, they were either given no food or fed a casein-based low-iron diet obtained from Nutritional Biochemicals Company. Corresponding control animals were either fasted for three days or were fed their normal diet of Wayne Medicated Rabbit Chow (Allied Mills Inc., Chicago, Ill.). Iron influxes were detemined 72 h after bleeding. No difference could be discerned between fasted or fed animals.

Iron-loaded anemics

Some rabbits were given 150 mg of iron-dextran (Imferon, Lakeside Laboratories, Milwaukee, Wisconsin) by intramuscular injection 24 h after bleeding. They were fed the same diet as the anemic rabbits and killed 72 h after bleeding.

RESULTS

The uptake of iron as a function of time for groups of control and anemic animals is plotted in Fig. 1. The data for each group describe a line which extrapolates to a positive intercept on the ordinate. This non-zero intercept indicates a biphasic uptake of iron by the tissue[2]: an initial, rapid uptake of tracer, followed by the slower, linear uptake observed from 0.5 to 4 min. Clearly, the lines for control and anemic

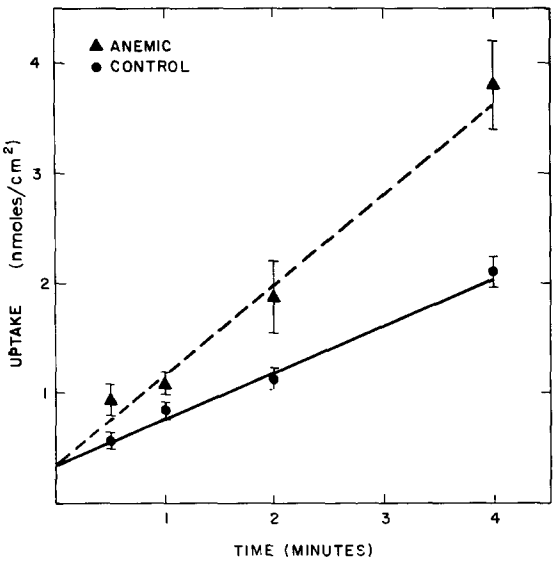

Fig. 1. Time course of iron uptake in normal and anemic rabbits. Values are means ± S. E. of 18–24 determinations on controls and 8–11 determinations on anemic rabbits. The lines were determined by least-squares regression analysis.

animals have significantly different slopes, 0.43 ± 0.09 and 0.81 ± 0.17 nmoles/cm² per min, respectively, yet both intersect the ordinate at points that do not differ significantly. This common intercept may be attributable to non-specific rapid binding of iron by the mucosal membrane or the fuzzy coat in view of the fact that it does not vary significantly under conditions that are known to enhance iron absorption (see Table I). The slower process probably represents movement across the brush border into the cells so that the slopes of the lines in Fig. 1 provide a measure of unidirectional influx[2].

TABLE I

IRON UPTAKE

Averages ± S. E. Number of experiments given in parentheses.

Experimental group	*Slope* (*nmoles/cm² per min*)	*Intercept* (*nmoles/cm²*)	*Hematocrit* (%)
Controls	0.51 ± 0.05 (44)	0.39 ± 0.07	44 ± 1 (12)
Anemic	0.85 ± 0.09 (30)	0.26 ± 0.10	30 ± 1 (11)
Imferon-anemic	0.46 ± 0.05 (20)	0.33 ± 0.09	31 ± 3 (5)

Table I shows the averages of the influx of ^{59}Fe across the brush border of duodenum from normal, anemic, and iron-loaded anemic rabbits; also shown are representative hematocrits. These values were obtained from measurements of uptake after 1 and 4 min exposure of the mucosal surface alone to ^{59}Fe assuming the linear time course illustrated in Fig. 1. The initial rapid uptakes of ^{59}Fe given by the calculated intercepts do not differ significantly. In contrast, unidirectional iron influx

in anemic animals is significantly greater than that in the control group. Anemic animals had a mean hematocrit of 30 ± 1,72 % of the control value of 44 ± 1. Additional data on blood iron were obtained for two control and four anemic animals: the serum iron concentration of the anemic animals, 82± 12 μg per 100 ml serum, was significantly lower than that of the normal rabbits, 130 μg per 100 ml serum. The percent saturation of transferrin in anemic rabbits was 23 ± 3, *versus* 33 ± 2 for controls. The average total iron binding capacity of anemic animals did not differ significantly from that of the controls.

Iron influx in bled rabbits that were treated with Imferon one day after bleeding averaged 0.46 ± 0.05 nmoles/cm² per min, a value only 53 % that of the anemic animals but comparable to that of the controls. The Imferon-treated rabbits were still anemic, having a mean hematocrit of 31 ± 3, a value that does not differ significantly from the hematocrits of anemic rabbits that did not receive Imferon.

DISCUSSION

Although it is well established that iron absorption by the duodenum is enhanced under conditions of iron deficiency, the cellular mechanisms of normal as well as stimulated iron absorption are, as yet, poorly understood. Previous studies, using both *in vivo* and *in vitro* preparations of rat duodenum, have suggested that specific transfer mechanisms are responsible for the movement of iron from the lumen across the mucosal membrane into the cell and for the subsequent transfer from the cell across the serosal membrane into the plasma or serosal solutions[5]. Enhanced iron absorption could result from a primary effect of iron deficiency on the transfer mechanism at the mucosal border or a primary effect on the serosal transfer mechanism or both. Increased activity on the part of a serosal mechanism alone would be expected to increase net movement across the brush border secondarily, through a decrease in the unidirectional efflux from the cell into the lumen; an increase in the unidirectional influx from the lumen across the brush border into the cell need not occur.

GREENBERGER *et al.*[6] have examined ^{59}Fe uptake by isolated brush borders from rat proximal and distal small intestine in an attempt to evaluate the role of the microvillus membrane in the regulation of iron absorption. Greater ^{59}Fe uptake was observed using brush borders from proximal small intestine than from more distal regions. Iron loading depressed uptake by proximal brush borders but had no effect on uptake by distal brush borders. In contrast, iron depletion enhanced ^{59}Fe uptake by distal brush borders but had no effect on uptake by proximal brush borders. Although these observations suggest that brush border mechanisms are involved in the regulation of iron absorption, as pointed out by GREENBERGER *et al.*[6], the relation between iron binding by isolated microvilli and transport across the brush border is unclear.

The present results demonstrate conclusively that iron deficiency resulting from controlled hemorrhage brings about a highly significant increase in the unidirectional influx of iron across the brush border of rabbit duodenum. Iron-loading anemic animals abolished this increase, suggesting that it is a consequence of diminished iron stores rather than anemia *per se*. These experiments do not exclude the possibilities that there is, in addition, (a) a decrease in the unidirectional efflux out

of the cell into the lumen and/or (b) an additional effect of iron deficiency on the activity of a carrier mechanism located at the serosal membranes. However, these results strongly suggest that the stimulation of intestinal iron absorption by iron deficiency involves the mechanism(s) responsible for entry across the mucosal border and cannot be attributed to an effect on the serosal transfer mechanism alone.

ACKNOWLEDGEMENTS

This project was supported by research grants from the U. S. Public Health Service, National Institutes of Health (AM-13744) and the American Heart Association (70-633). S.G.S. was the recipient of a Research Career Development Award from the National Institute of Arthritis and Metabolic Diseases (AM-9013).

REFERENCES

1 W. H. CROSBY, *Iron Absorption*, in *The Handbook of Physiology*, Section 6, *The Alimentary Canal*, Vol. 3, *Intestinal Absorption*, American Physiological Society, Washington, D. C., 1968, pp. 1553–1570.
2 S. G. SCHULTZ, P. F. CURRAN, R. A. CHEZ AND R. E. FUISZ, *J. Gen. Physiol.*, 50 (1967) 1241.
3 W. T. CARAWAY, *Clin. Chem.*, 9 (1963) 188.
4 B. BROZOVICH, *J. Clin. Path.*, 21 (1968) 183.
5 J. G. MANIS AND D. SCHACHTER, *Am. J. Physiol.*, 203 (1962) 73.
6 N. J. GREENBERGER, S. P. BALCERZAK AND G. A. ACKERMAN, *J. Lab. Clin. Med.*, 73 (1969) 711.