ELSEVIER

Journal of the Neurological Sciences 134 (Suppl.) (1995) 45–51

JOURNAL OF THE
NEUROLOGICAL
SCIENCES

Effects of diet and development upon the uptake and distribution of cerebral iron

Andrew J. Dwork *

Department of Neuroscience, Division of Neuropathology, New York State Psychiatric Institute, Unit 62, 722 West 168th Street, New York, NY 10025, USA
Departments of Pathology (Division of Neuropathology) and Psychiatry, College of Physicians and Surgeons of Columbia University, New York, NY, USA

Accepted 14 July 1995

Abstract

In order to determine whether iron sequestered by the rat brain during the third week of postnatal life could be mobilized by subsequent dietary iron deficiency (ID), iron-59 (^{59}Fe) was administered to rats at 2 weeks of age. The animals were placed on an ID or a control diet from age 4 through 8 weeks and killed by perfusion. Brain radioactivity was identical for both groups, and autoradiography revealed no differences in the distribution of radioactivity. Thus, neither the sequestration of cerebral iron acquired at age 2 weeks nor its subsequent redistribution was affected by ID. Since ID beginning after age 3 weeks reportedly produces a cerebral iron deficit that is in part reversible, an attempt was made to determine whether ^{59}Fe administered after ID was preferentially delivered to any brain region. Rats were placed on an ID or a control diet from age 3 through 7 weeks and then injected with ^{59}Fe, placed on a normal diet, and killed 2 weeks later. Thre was no difference between groups in amount or distribution of brain ^{59}Fe, except in the choroid plexus, which was more radioactive in the ID rats than in the controls. This finding may represent a mechanism by which the choroid plexus buffers the brain against rapid rises in plasma iron content.

Keywords: Autoradiography; Brain; Choroid plexus; Hemochromatosis; Iron; Iron deficiency; Rat

1. Introduction

It has been recognized for nearly two decades that, in the rat, cerebral uptake of iron peaks during the third week of postnatal life (Dallman and Spirito, 1977). Peak uptake coincides with the development of transferrin (Tf)-immunoreactive oligodendrocytes, which first appear in gray matter at age 15 days, and 1 week later have attained a distribution close to that found in the adult brain (Connor and Fine, 1987). Cerebral iron acquired during this period is not readily exchanged with extracerebral iron (Dallman and Spirito, 1977). Early dietary iron deficiency (ID) leads to a permanent deficit in cerebral iron (Dallman et al., 1975; Weinberg et al., 1979, 1980; Youdim et al., 1980; Findlay et al., 1981; Ben-Shachar et al., 1986), while lack of brain iron produced by ID after weaning at age 21 days is at least in part reversible. It thus appears that there is a temporal window of opportunity for the acquisition of iron by the brain, and that cerebral iron acquired during this period remains sequestered within the brain, although it probably undergoes redistribution (Dwork et al., 1990).

The current experiments were undertaken to determine whether ID occurring shortly after the crucial period of cerebral iron uptake could (a) cause release of any cerebral iron acquired during this period, or (b) create a reversible cerebral deficit that could subsequently be corrected by supplementary iron.

2. Material and methods

All animal experiments described here were approved by an Institutional Animal Care and Use Committee.

Male Sprague-Dawley rats were weaned at 21 days. Iron-deficient or control diet (Teklad, Madison, WI, USA) was administered for 4 weeks, either from days 21 to 49 or from days 28 to 56 of animal age. The ID diet is based on casein (Dallman et al., 1975) and is essentially the American Institute of Nutrition "AIN-76" diet (American Institute of Nutrition, 1976) minus iron salts and cellulose. The two lots used for these experiments contained 2.2 and 3.5

* Tel.: (212) 960-5563; Fax: (212) 960-5769

SSDI 0022-510X(95)00207-3

mg iron/kg food, respectively. The control diet was identical but contained added iron of 48 mg/kg. Both groups were given deionized distilled water ad libitum. Weaned animals were kept in cages with screened floors in order to prevent coprophagia. When not on ID or control diets, animals were fed standard laboratory chow and tap water.

[^{59}Fe]Cl_3 (Amersham, Arlington Heights, IL, USA), 3–20 Ci/g in 0.1 N HCl, was either injected directly into the peritoneal cavity or diluted with 2 volumes of 0.1 M citrate buffer (pH 6.0) for intravenous (i.v.) injection into the exposed jugular vein. The animals receiving i.v. injections were anesthetized by methoxyflurane.

Death by cardiac perfusion with heparinized, phosphate-buffered saline followed by phosphate-buffered formalin, gamma counting, autoradiography, and image analysis were all as described previously (Dwork et al., 1990), except that imaging and analysis was performed on a Macintosh IIfx computer using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from ftp://zippy.nimh.nih.gov). To correct for variations in absorbed dose of ^{59}Fe, tissue radioactivity was normalized to blood radioactivity measured 48 h after intraperitoneal (i.p.) injection or 2 min after i.v. injection (Dwork et al., 1990). All measurements of radioactivity were corrected for isotopic decay.

Blood hemoglobin was measured by absorbance at 540 nm after dissolving the blood in Drabkin's solution (Fairbanks and Klee, 1986). Standards were prepared from rat hemoglobin (Sigma Chemical Co., St. Louis, MO, USA).

Neuropathologic examination was performed on the brain of a 51-year-old woman with hemochromatosis, mild mental retardation, early ovarian failure, and well-controlled grand mal seizures since youth. Serum iron was 189 μg/dl, and serum ferritin 3486 ng/ml. The brain was fixed in 10% formalin for approximately 2 weeks. After gross examination, sections were embedded in paraffin and stained with hematoxylin and eosin. Selected sections were stained with Mallory's iron stain.

3. Results

Iron deficiency from age 28 to 56 days produced no change in the amount or distribution of cerebral ^{59}Fe following i.p. injection at age 14–15 days (Fig. 1). Despite a 57% reduction in hemoglobin, blood radioactivity was increased and liver and spleen radioactivity decreased, representing increased mobilization of reticuloendothelial stores for hematopoiesis (Table 1).

In the second experiment, intravenous ^{59}Fe was administered immediately after ID or control diets were given from the ages of 21–49 days. The animals were then fed regular lab chow for two weeks, before death at the age of 9 weeks. There was no difference in total cerebral uptake of ^{59}Fe although splenic uptake in ID rats was approxi-

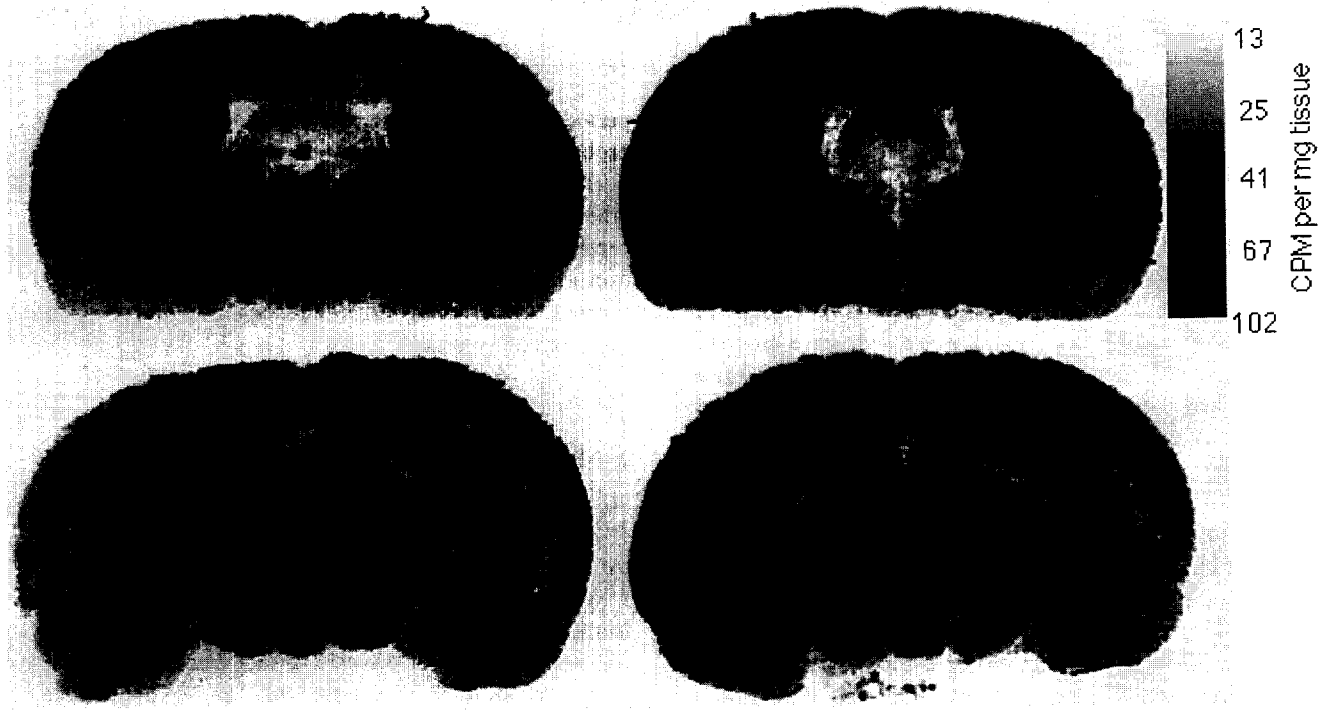

Fig. 1. Computer-generated images of autoradiograms of rat brains. Coronal sections at levels of globus pallidus (top) and substantia nigra (bottom). Rats were injected i.p. with ^{59}Fe at age 14 days, placed on ID (left) or control diets (right) at age 28 days, and killed at age 56 days. The available dose of ^{59}Fe for the rat on the right, as estimated from blood radioactivity 48 h later, was 81% greater than that received by the rat on the left. This is reflected in greater total radioactivity of brain on the right, but relative distributions are similar. Scale applies to all images, which were on a single autoradiogram.

Table 1
Radioactivity in brain, blood, liver, and spleen after the i.p. administration of ^{59}Fe to rats with subsequent iron deficiency. Blood hemoglobin (Hb) is given as g/dl at the indicated ages. ^{59}Fe in blood, liver, and spleen was determined at age 8 weeks, following i.p. injection of the labeled iron at age 14–15 days. Animals were on an ID or control diet from the age of 4 weeks. ^{59}Fe values are given as ratios to the radioactivity in 1 μl blood drawn 48 h after the injection. $n = 8$ per group for brain, 6 per group for 4-week hemoglobin, 11 per group for other measures. Values are mean ± SE.

	Iron deficient	Control	p
Brain	32.3 ± 1.6	32.0 ± 1.5	0.9
Blood (μl)	0.19 ± 0.01	0.15 ± 0.01	0.02
Liver (whole)	138 ± 13.7	205 ± 27.3	0.04
Liver (mg)	0.017 ± 0.001	0.026 ± 0.002	< 0.001
Spleen (whole)	58.8 ± 3.4	97.8 ± 15.5	0.03
Spleen (mg)	0.092 ± 0.007	0.150 ± 0.019	0.01
Hb at 4 wks	14.5 ± 0.7	14.9 ± 0.7	0.7
Hb at 8 wks	8.5 ± 0.6	19.7 ± 0.6	< 0.001

Table 2
Radioactivity in brain, blood, liver, and spleen after the intravenous administration of ^{59}Fe to rats with prior iron deficiency. Blood hemoglobin (Hb) is given as g/dl at the indicated ages. ^{59}Fe in brain, blood, liver, and spleen was determined at age 9 weeks, following ID or control diet, respectively, from age 3 weeks through 7 weeks and i.v. injection of ^{59}Fe at age 7 weeks. ^{59}Fe values are given as ratios to radioactivity in 1 μl blood drawn 2 min after injection. $n = 7$ ID animals and 4 controls for brain, 10 ID animals and 8 controls for other measures. Values are mean ± SE.

	Iron deficient	Control	p
Brain	17.0 ± 1.0	17.3 ± 1.8	0.9
Blood (μl)	0.79 ± 0.07	0.76 ± 0.04	0.8
Liver (whole)	1341 ± 158	1668 ± 83	0.09
Liver (mg)	0.18 ± 0.02	0.21 ± 0.02	0.3
Spleen (whole)	364 ± 52	180 ± 22	0.009
Spleen(mg)	0.54 ± 0.56	0.28 ± 0.03	0.001
Hb at 3 wks	11.5 ± 0.5	11.4 ± 0.6	0.8
Hb at 7 wks	7.6 ± 0.7	20.1 ± 0.7	< 0.001
Hb at 9 wks	19.2 ± 1.0	20.8 ± 1.2	0.3

mately double that in control rats (Table 2). Cerebral autoradiography was performed on three animals from each group, and also on several animals treated identically but who received either i.p. injections or attempted i.v. injections that were inadvertently delivered primarily to soft tissues. No differences were seen between ID or control animals, except for the choroid plexus, which was considerably more prominent in the autoradiograms of the ID rats (Fig. 2). This result was quantified by comparing radioactivity of choroid plexus and gray matter on the autoradiograms. Ratios were determined for each section in which lateral or third ventricular choroid plexus was visualized, and an average ratio was obtained for each animal. The ratios were 51% higher for the ID animals (ID 4.41 ± .37 (mean ± SE), $n = 7$; control 2.69 ± 0.38, $n = 8$; $p = 0.009$ by the two-tailed t-test following log transformation). One ID brain and one control brain were exposed on separate autoradiograms; the other autoradiograms all contained at least one ID animal and one control animal. Stepwise regression showed no significant effect by autoradiogram or method of injection. Two pairs of ID and control animals were treated in parallel, injected i.v., and exposed on the same autoradiographic film. The results for these pairs were similar to those for the whole group. The choroid plexus/gray matter ratio was 71% and 46% higher in the ID member of the two pairs. For these two pairs, it was also appropriate to compare the ratio of choroid plexus radioactivity to 2-min post-injection blood radioactivity. This ratio was 123% and 124% higher for the ID member of each pair, confirming that the increased choroid plexus/brain ratio in ID animals was due to enhanced uptake or retention of iron in the choroid plexus, rather than decreased uptake or retention by the brain.

Analysis of the autoradiograms probably underestimates the difference between ID and control conditions, since choroid plexus could be identified on the autoradiograms and sampled only when it was more radioactive than the surrounding parenchyma, and this was the case in many more sections of the ID brains than of the control brains, particularly after i.p. injections.

A qualitative comparison was made of the autoradio-

Fig. 2. Computer-generated images of autoradiograms from rats placed on ID (left) or control diets (right) from age 21 to 49 days, injected i.v. with ^{59}Fe at age 50 days, and killed at age 63 days. Grey matter radioactivity is similar in both brains, while choroid plexus is more radioactive in brain on left (ID). Scale applies to both images, which were on a single autoradiogram.

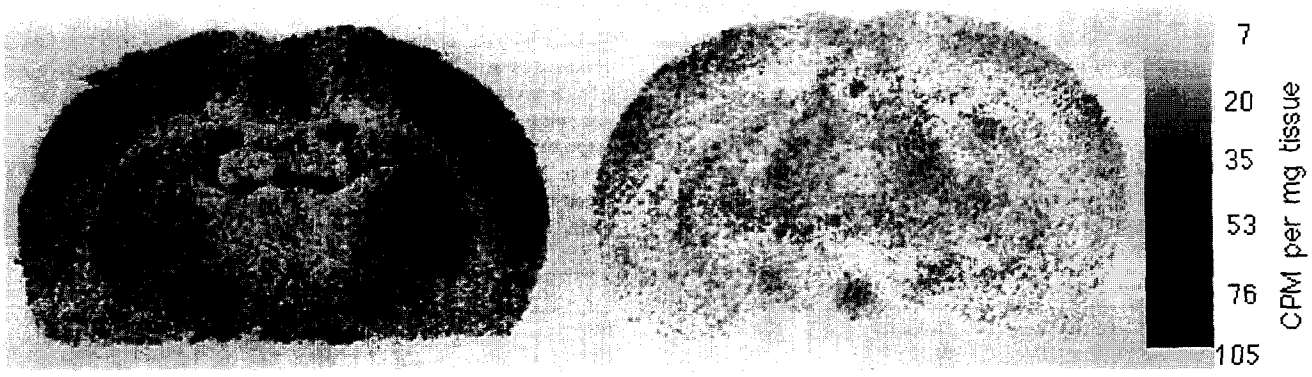

Fig. 3. Computer-generated images of autoradiogram of normal rat brains after the i.p. injection of ^{59}Fe at age 28 days and death at age 42 days. Coronal sections at levels of globus pallidus (left) and substantia nigra (right). The choroid plexus, seen in the image on the left, is far more radioactive than gray matter, which shows fairly uniform radioactivity.

grams of brains of normal rats injected i.p. with ^{59}Fe at ages 14–15 or 28 days and killed 2 weeks later. When ^{59}Fe was administered i.p. at age 14–15 days, choroid plexus was not appreciated on autoradiography, and the ventricles appeared virtually devoid of radioactivity (Dwork et al., 1990). When ^{59}Fe was administered at age 4 weeks, the choroid plexi were by far the most radioactive structures in the brain (Fig. 3).

Gross examination of the fixed brain of the woman with hemochromatosis was unremarkable except for orange discoloration of the olfactory bulbs and the choroid plexi. Microscopic examination of hematoxylin and eosin-stained slides revealed brown, granular pigment in the choroid plexi, the subpial layer of the olfactory bulbs, and to a much lesser extent, in the area postrema. Mallory iron stain confirmed the presence of iron in this pigment. In the choroid plexi, the iron was primarily in epithelial cells. Many contained abundant deposits, while others were unstained (Fig. 4). No iron deposits were labeled in the gray or white matter. Microscopic examination was otherwise unremarkable except for mild cerebellar atrophy.

4. Discussion

We demonstrated previously that virtually all ^{59}Fe acquired by the brain after i.p. injection at age 15 days is

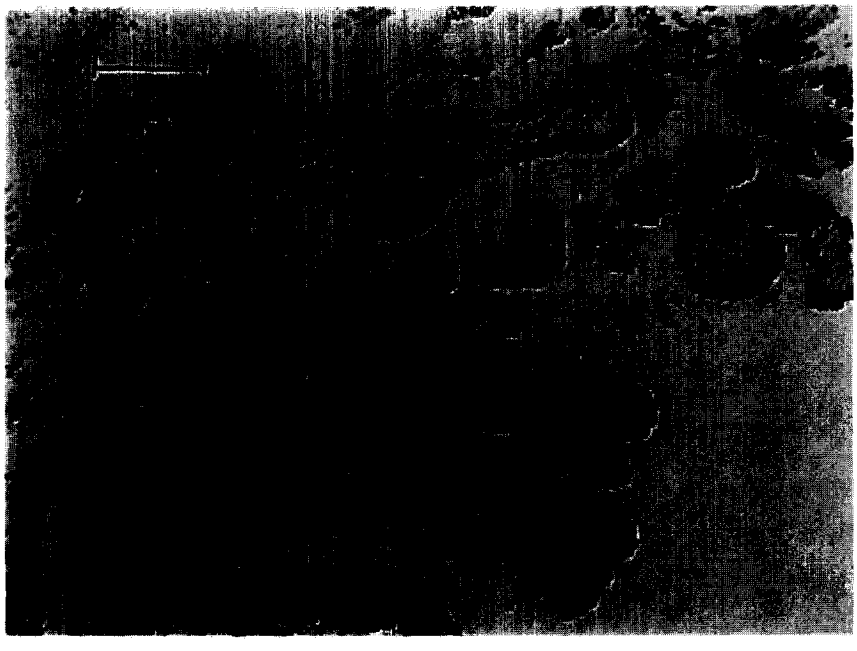

Fig. 4. Mallory iron stain of fourth ventricular choroid plexus from the autopsy of a 51-year-old woman with hemochromatosis (serum iron 189 μg/dl, serum ferritin 3486 ng/ml). Extensive iron deposits are present in many but not all choroid plexus epithelial cells. Section was counterstained by nuclear fast red. Magnification marker = 100 μm.

taken up within 2 days of administration and remains there at least until age 72 days (Dwork et al., 1990). The present study demonstrates that this iron is not mobilized from the brain even during severe systemic iron deficiency. Total cerebral radioactivity at age 56 days was essentially identical in animals placed on ID or control diet from age 28 days. The value for both groups was the same as that obtained in a previous set of experiments for the brains of 17-, 23-, 30- 43- or 72-day-old rats that had received i.p. ^{59}Fe at age 15 days (Dwork et al., 1990). Furthermore, autoradiograms of the brains of ID and control rats were identical, demonstrating no localized loss of early-acquired iron that might be too small to be detected by whole brain gamma counting. Thus, the sequestration of cerebral iron acquired at age 15 days appears to be fairly complete.

The sequestration of cerebral iron acquired at that age is further demonstrated by the failure of iron chelating agents to reduce cerebral ^{59}Fe when administered 2 or 24 h after the labeled iron (Crowe and Morgan, 1994). However, two studies report contrary findings. Dallman and Spirito (1977) performed i.p. injections of ^{59}Fe at age 15 days and removed about 1 ml of blood by phlebotomy at ages 21, 25, and 28 days, while simultaneously administering supplemental iron intramuscularly (i.m.). The brains of these rats killed at the age of 50 days contained 33% less radioactivity that those of rats killed at age 21 days. There was no subsequent loss up to age 150 days. It thus may be that phlebotomy during the fourth week of life mobilizes iron more effectively than does dietary deficiency beginning in the fifth week of life. Crowe and Morgan (1992) reported no loss of cerebral radioactivity for the first 28 days after i.v. injection at age 18 days but a 45% loss between ages 46 and 74 days. These results thus differ from ours, which showed no loss of cerebral radioactivity. They are compatible with those of Dallman and Spirito (1977) only if the entire loss of brain radioactivity occurs between the ages of 46 and 50 days, which seems unlikely. Some of the differences may be attributable to the later time and i.v. route of injection used by Crowe and Morgan (1992). However, serum ^{59}Fe drops even more rapidly after i.v. injection than after i.m. injection. Presumably, all of the cerebral ^{59}Fe was acquired by the brain very soon after injection, and it is surprising that it would remain perfectly sequestered for 28 days and then begin to disappear.

Cerebral uptake of iron is assumed to be primarily by receptor-mediated endocytosis of Tf-bound iron in capillary endothelial cells, which are rich in Tf receptors (Jeffries et al., 1984). The mechanism by which iron leaves the endothelial cells and becomes incorporated into brain parenchyma is unknown, and it is unclear whether Tf is involved in this process (Fishman et al., 1987; Taylor et al., 1991; Crowe and Morgan, 1992; Roberts et al., 1992). It seems likely that plasma Tf is mostly recycled back to the luminal side of cerebral capillary endothelial cells. Transferrin synthesized in the brain may be involved in the intracerebral transport of iron, once the iron has passed to the abluminal side of the capillary endothelium.

The role of the choroid plexus in brain iron metabolism is even less well understood than that of the capillary endothelium. Choroid plexus epithelial cells synthesize transferrin (Bloch et al., 1985) and probably secrete the protein into the cerebrospinal fluid (CSF) (Dziegielewska et al., 1985; Aldred et al., 1987; Thomas et al., 1989). Some transfer of plasma Tf to CSF does occur (Crowe and Morgan, 1992) though the site of transport could be either the choroid plexus or the walls of blood vessels. Since the accumulation of plasma-derived Tf is more rapid in brain extracellular fluid than in CSF (Crowe and Morgan, 1992), it is clear that the plasma-derived Tf in brain extracellular fluid is not acquired primarily through the choroid plexus. Crowe and Morgan (1992) demonstrated that in 15-, 21-, or 63-day-old rats, the accumulation of ^{59}Fe by choroid plexus exceeded that of labeled Tf at 0.5–6 h after the i.v. injection of ^{59}Fe-labeled Tf. They found no change in the accumulation of either labeled Tf or ^{59}Fe in choroid plexus over this 5.5-h period. This observation differs from the results in brain proper (including endothelium), where accumulation of labeled Tf plateaued within 15 min of i.v. injection, while ^{59}Fe continued to increase for at least 6 hours after i.v. injection (Taylor et al., 1991). The plasma half-life of ^{59}Fe in these experiments was ~30 min (Taylor et al., 1991). These observations imply that choroid plexus turns over iron more rapidly than does the brain proper. It seems logical to hypothesize that this rapid turnover of iron is facilitated by choroid plexus-derived Tf, which could aid in the delivery of iron to CSF or in its return to plasma. In the adult rat, i.v. infused ^{59}Fe appears more rapidly in brain than in CSF (Ueda et al., 1993). While this result supports the notion that the major route of entry of iron into brain is not via choroid plexus, it does not rule out the possibility that some iron, possibly bound to choroid plexus-synthesized Tf, is secreted into CSF from that source.

Several observations suggest that the choroid plexus may be involved in keeping iron out of the brain. As noted above, there was greater uptake by choroid plexus of ^{59}Fe injected into 7-week-old ID rats than into 7-week-old control rats, despite equal uptake by the brain. The measurements were made 2 weeks after injection. If the primary function of the choroid plexus with respect to iron were transport into the brain, and if this mechanism were regulated by brain iron status, one would expect more rapid transport of iron into ID brain than into control brain, leaving less iron in the choroid plexus of the ID animals. Instead, administered iron is trapped or retained by ID choroid plexus with greater efficiency than by control choroid plexus. This could represent a protective mechanism by which the choroid plexus, whose capillary endothelium lacks the tight junctions of the blood–brain barrier, buffers the brain in the event of a rapid increase in serum iron. Since the synthesis of Tf by choroid plexus is

not affected by ID (Idzerda et al., 1986), it is conceivable that during ID, a lower saturation of choroid plexus Tf enhances the efficiency with which this structure absorbs or retains iron.

The case of human hemochromatosis provides further evidence that the choroid plexus may help protect the brain against iron overload. The choroid plexus epithelial cells contain abnormal deposits of iron, while no such deposits are seen in brain parenchyma. These deposits were easily appreciated on sections stained routinely with hematoxylin and eosin. In the author's experience, they are not found in the absence of systemic iron overload.

Autoradiography after i.p. administration of ^{59}Fe at age 14–15 days, when cerebral uptake of iron is high, does not demonstrate the choroid plexus, and the ventricles appear virtually devoid of radioactivity. On the other hand, if ^{59}Fe is administered at age 4 weeks, when cerebral iron uptake is much lower, the choroid plexi are the most radioactive structures in the brain at two weeks after the injection. This developmental change further suggests that choroid plexus retains iron in an inverse relationship to cerebral uptake.

Autoradiography is not the ideal method for measuring radioactivity in the choroid plexus. Dissection and gamma counting would yield more accurate results. The reported findings in the choroid plexus are serendipitous. Based on reports that ID after the third week of life produces a reversible deficit in cerebral iron (Ben-Shachar et al., 1986), these studies sought to determine whether this process involved cerebral iron deposits that are anatomically distinct from those acquired during the third week of life. From this point of view, the results were negative. Aside from the choroid plexus, there were no generalized or localized differences between ID and control rats. Possibly, this result was caused by the administration of ^{59}Fe at the end of the period of ID, rather than while recovery from ID was already in progress. The fate of supplemental iron following ID might be better studied by continuing the animals on an ID diet while administering therapeutic dose of tagged iron by i.m. injection.

In contrast to the present results, Taylor et al. (1991) were able to demonstrate increased cerebral uptake of ^{59}Fe by ID rats at various ages from 15 to 63 days ($\sim$ 100% increase at age 21 days, declining to $\sim$ 25% at age 63 days). However, ID in these animals commenced in utero and continued throughout life, which produces a much more severe deficit in cerebral iron than does ID after weaning (Findlay et al., 1981). The study of Taylor et al. (1991) did not address the uptake of iron by the choroid plexus nor the distribution of cerebral iron acquired under these circumstances.

Studies of the roles of ID and of choroid plexus in cerebral iron metabolism are only beginning. Much more information can be gained by examining the fate of ^{59}Fe pulses. These investigations will have to be quite detailed in order to elucidate what will probably be a variety of transport mechanisms changing continuously through early development.

Acknowledgements

This work was supported by MH 43639.

References

Aldred, A.R., P.W. Dickson, P.D. Marley and G.S. Schreiber (1987) Distribution of transferrin synthesis in brain and other tissues in the rat. J. Biol. Chem., 262: 5293–5297.

American Institute of Nutrition (1976) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J. Nutr., 107: 1340–1348.

Ben-Shachar, D., R. Ashkenazi and M.B.H. Youdim (1986) Long-term consequences of early iron-deficiency on dopaminergic neurotransmission in rats. Int. J. Dev. Neurosci., 4: 81– 88.

Bloch, B., T. Popovici, M.J. Levin, D. Tuil and A. Kahn. (1985) Transferrin gene expression visualized in oligodendrocytes of the rat brain using in situ hybridization and immunohistochemistry. Proc. Natl. Acad. Sci. USA, 82: 6706–6710.

Connor, J.R. and R.E. Fine. (1987) Development of transferrin-positive oligodendrocytes in the rat central nervous system. J. Neurosci. Res., 17: 51–59.

Crowe, A. and E.H. Morgan (1992) Iron and transferrin uptake by brain and cerebrospinal fluid in the rat. Brain Res., 2: 8–16.

Crowe, A. and E.H. Morgan (1994) Effects of chelators on iron uptake and release by the brain in the rat. Neurochem. Res., 19: 71–76.

Dallman, P.R., M.A. Simes and E.C. Manies (1975) Brain iron: persistent deficiency following short-term iron deprivation in the young rat. Br. J. Haematol., 31: 209–215.

Dallman, P.R. and R.A. Spirito (1977) Brain iron in the rat: Extremely slow turnover in normal rats may explain long-lasting effects of early iron deficiency, J. Nutr., 107: 1075–1081.

Dwork, A.J., G. Lawler, P.A. Zybert, M. Durkin., M. Osman, N. Willson and A. Barkai (1990) An autoradiographic study of the uptake and distribution of iron by the brain of the young rat. Brain Res., 518: 31–39.

Dziegielewska, K.M., C.A.N. Evans, H. New, M.L. Reynolds and N.R. Saunders (1985) Synthesis of plasma proteins by rat fetal brain and choroid plexus. Int. J. Dev. Neurosci., 2: 215–222.

Fairbanks, V.F. and G.G. Klee (1986) Biochemical aspects of hematology. In: Teitz, N.W. (Ed.), Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, pp. 1495–1588.

Findlay, E., K.T. Ng,, R.L. Reid and S.M. Armstrong (1981) Developmental changes in body iron stores following prolonged iron depletion in the rat. Physiol. Behav., 27: 1097–1102.

Fishman, J.B., J.B. Rubin, J.V. Handrahan,, J.R. Connor and R.E. Fine (1987) Receptor-mediated transcytosis of transferrin across the blood–brain barrier. J. Neurosci. Res., 18: 299–304.

Idzerda, R.L., H. Huebers, C.A. Finch and G.S. McKnight (1986) Rat transferrin gene expression: tissue-specific regulation by iron deficiency. Proc. Natl. Acad. Sci. USA, 83: 3723–3727.

Jeffries, W.A., M.R. Brandon, S.V. Hunt, A.F. Williams, K.C. Gatter and D.Y. Mason (1984) Transferrin receptor on endothelium of brain capillaries, Nature, 312: 162–163.

Roberts, R., A. Sandra, G.C. Siek, J.J. Lucas and R.E. Fine (1992) Studies of the mechanism of iron transport across the blood–brain barrier. Ann. Neurol., 32 (Suppl.): S43-S50.

Taylor, E.M., A. Crowe and E.H. Morgan (1991) Transferrin and iron uptake by the brain: effects of altered iron status. J. Neurochem., 57: 1584–1592.

Thomas, T., G. Schreiber and A. Jaworowski (1989) Developmental patterns of gene expression of secreted proteins in brain and choroid plexus. Dev. Biol., 134: 38–47.

Ueda, F., K.B Raja, R.J. Simpson, I.S. Trowbridge and M.W. Bradbury (1993) Rate of ^{59}Fe uptake into brain and cerebrospinal fluid and the influence thereon of antibodies against the transferrin receptor. J. Neurochem., 60: 106–113.

Weinberg, J., S. Levine and P.R. Dallman (1979) Long-term consequences of early iron deficiency in the rat. Pharmacol. Biochem. Behav., 11: 631–638.

Weinberg, J., P.R. Dallman and S. Levine (1980) Iron deficiency during early development in the rat: behavioral and physiological consequences. Pharmacol. Biochem. Behav., 12: 493–502.

Youdim, M.B.H., A.R. Green, M.R. Bloomfield, B.D. Mitchell, D.J. Heal and D.G. Grahme-Smith (1980) The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. Neuropharmacology, 19: 259–267.