Iron and the brain: neurotransmitter receptors and magnetic resonance spectroscopy

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Earlier studies show that in iron deficiency with anaemia and in latent iron deficiency neurotransmitters are altered. The changes induced in the fetal brain are irreversible on rehabilitation. The important alterations in glutamate metabolism in latent iron deficiency stimulated studies on gamma aminobutyric acid and glutaminate receptors. It was observed that binding of ^{3}H-muscimol at pH 7·5 and 1 mg protein/assay increased significantly in synaptic vesicular membranes and under similar conditions ^{3}H-glutamate binding showed reduction. Thus iron deficiency played a role in both excitatory and inhibitory neurotransmitter receptors. To elucidate the role of body iron status on the brain, anaemic children with thalassemia and iron deficiency were subjected to 'magnetic resonance spectroscopy' of globus pallidus, caudate and dentate nuclei and there was no change in iron content. The concentrations of creatinine and aspartate increased, with lowering of choline content. The findings were similar in thalassemia as well as iron deficiency anaemia, suggesting that in anaemia changes operate through reduced oxygen availability.

Iron: Deficiency: Neurotransmitters

Studies from different parts of India in the last two decades have shown that nutritional anaemia, usually due to iron deficiency, affects 60–90 % of the population, particularly pregnant women, young children and adolescents (WHO, 1992; ICMR, 1989; ICMR, 1992; Gomber *et al.* 1998). Our earlier studies in pregnancy anaemia demonstrated that in maternal hypoferremia, the transfer of iron to fetus is at a gradient but proportionate to maternal iron level (Singla *et al.* 1978; Singla *et al.* 1979), the fetal iron stores are low (Singla *et al.* 1985), the concentration of iron in breast milk is higher (Franson *et al.* 1985) and the treatment of anaemia with iron folate improves birth weight (Agarwal *et al.* 1991).

Felt & Lozoff (1996) reported that in iron deficiency anaemic rat mothers despite rehabilitation by iron as early as mid-gestation, the brain iron content remained low in the offspring and behavioural changes persisted at 3 months of age. Iron deficiency anaemia reduced brain iron, but brain enzymes were resistant to change, although activities of aminobutyric acid transaminase and glutamate decarboxylase, decreased (Youdim & Green, 1977; Youdim *et al.* 1989). Iron deficiency anaemia changes the dopamine, serotonin and gamma aminobutyric acid/(GABA) systems (Beard *et al.* 1993). Further, binding receptors of dopamine D2 (^{3}H-spiperone) decrease and GABA (^{3}H-muscimol) increases in the brain (striatum and cortex) of iron deficient anaemic rats. Nelson *et al.* (1997) demonstrated that in iron deficiency anaemia the extracellular level of dopamine increased in the caudate–putamen brain area, but normalized on rehabilitation and that the elevation of dopamine or other neurotransmitters was not due to anaemia *per se*. In earlier studies alterations in 5-hydroxytryptophan, dopamine and norepinephrine were observed (Ashkenazi *et al.* 1982; Ben-Shachar *et al.* 1986). The above studies were mainly limited to the effects of iron deficiency anaemia on alterations in various brain neurotransmitters.

Iron, as a micronutrient, is required for regulation of brain neurotransmitters by altering the pathway enzymatic system. To study iron as a micronutrient, a rat model was developed to create iron-deficiency (low hepatic iron) without change in haematocrit. Pregnant and lactating rats were fed on 30 mg iron/kg diet (normal need 250–300 mg) and post-weaning rats received 18–25 mg/kg (normal need 60–80 mg). The body, brain and liver weights reduced at around 14–21 days postnatally in rat pups (mothers maintained on low iron diet in pregnancy and lactation), without any change in brain and hepatic protein, DNA and RNA contents. The hepatic iron showed marked reduction; 40 % in 20 days fetus and 85 % by postnatal day 21. The fetal as well as weanling rat brain iron content decreased significantly and this alteration was irreversible on rehabilitation. In post weaning rats, iron content reduced irreversibly; in the corpus striatum by 32 %, midbrain 21 %, hypothalamus 19 %, cerebellum 18 %, cerebral cortex 17 % and in the hippocampus by 15 %, but there was no change in the medulla oblongata (Taneja *et al.*

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1990). The corpus striatum in iron deficiency showed lower zinc content, while calcium and copper content had increased. There was a decrease in zinc and an increase in copper in the cerebral cortex. Calcium content in the hypothalamus increased as well (Shukla et al. 1989a). Such heterogeneous brain region iron distribution in iron deficiency anaemia was observed by Hill (1988).

The latent iron deficient rats (pregnancy and lactation) show decrease in activities of GABA shunt enzymes, which do not normalize on rehabilitation of dams with an iron rich diet (Taneja et al. 1990). In contrast latent iron deficient post weaning rats show decrease in GABA shunt enzyme activities, which recover on rehabilitation (Taneja et al. 1986; Shukla et al. 1989b). These post weaning rats show irreversible reduction in whole brain: dopamine, norepinephrine and tyrosine (catecholamine metabolism) and in tryptophan, 5-hydroxy tryptophan and 5-hydroxy indoleacetic acid (5-hydroxy tryptamine metabolism). In the corpus striatum of these rats dopamine, homovanilic acid, monoaminooxidase, tyrosine and to some extent norepinephrine are reduced irreversibly (Shukla et al. 1989c; Shukla et al. 1989d). These are specific affects of iron deficiency, as in intrauterine as well as postnatal malnutrition; neurotransmitter changes partially recover on rehabilitation (Prasad et al. 1979; Prasad & Agarwal, 1980).

We briefly report the changes in GABA and glutamate receptors in latent iron deficient rats (Agarwal et al. unpublished)

Materials and methods

Female mice of Sprague–Dawley strain were used. They were kept in plastic cages with stainless steel mesh. Synthetic diets contained all necessary components, as described earlier (Shukla et al. 1989a; Shukla et al. 1989d). Radioligands were purchased from Amersham (UK). All fine chemicals of analytical grade were obtained from Sigma or Merck India.

Creation of iron-deficiency in rats. Female albino rats were used in the experiments. Weanling (21-day-old) rats weighing 40 ± 5 g were divided into two groups, control and experimental. The experimental group was maintained entirely on an iron-deficient synthetic diet containing 18–20 mg Fe/kg. The control group received the same diet supplemented with iron to 260 mg Fe/kg. Water was served ad libitum in iron-free feeding bottles. At least six animals were included in each group.

Both experimental and control groups were given diets for 2 months. The rats were killed by cervical dislocation.

Blood was collected in plain as well as EDTA vials. Brain and liver were dissected out, rinsed in saline, weighed and frozen at $-20°C$ until the time of processing.

Hematological analysis. Brain and liver non-haem iron was determined in 10 % homogenate (Hallgren, 1953). Haemoglobin (Crosby & Houchin, 1957) and micro-haematocrit (Guest & Siler, 1934) were determined by standard laboratory protocols.

Neurotransmitter receptors GABA and L-glutamic acid receptors in brain were estimated by radioligand binding assays in synaptic membranes prepared according to Hell et al. (1990). For inhibitory neurotransmitter receptors, ³H-muscimol, a GABA A agonist, was used. Binding of ³H-muscimol (specific activity 25 Ci/mmol) for GABA receptors was done by the method described by Seth et al. (1981). The assay was done in presence or absence of 1×10^4 mmol GABA. Incubation was done at 37°C for 30 min. At the end of incubation, 2 ml of chilled buffer was added and the incubation mixture immediately filtered through a glass fiber filter under vacuum. The filters were rinsed twice in buffer, dried and counted in a liquid scintillation counter. Specific binding of radioligand for each concentration was carried out in triplicate.

For excitatory neurotransmitter receptors, ³H-glutamate binding was performed according to Cross et al. (1986). Binding was carried out in 200-μl volume in microtiter plates. Optimum pH and protein concentration was determined and found to be 7·4 and 1 mg respectively. Binding was carried out at 37°C, for 30 minutes. After the incubation, reaction mixture was filtered through Whatman GFB glass fiber filter using a cell harvester (Hall & Thor, 1979). The filters were washed, dried and counted in a liquid scintillation counter.

Results

Eight weeks of iron deficiency did not significantly change the gross weight of rat brain and liver. There was no effect on haemoglobin and haematocrit. The non-haem iron in the liver and the brain decreased significantly ($P < 0·001$, Table 1).

GABA-receptors ³H-muscimol binding to synaptic membrane was dependent both on pH and concentration of protein. The assay was carried out at optimum pH (7·5) and protein concentration (1 mg/assay). Binding of ³H-muscimol increased by 193 % in membranes from iron-deficient rats as compared to controls (Table 2).

Glutamate receptors like muscimol, ³H-glutamate binding was also dependent on pH and concentration of membrane vesicles. There was significant reduction by

Table 1. Effect of iron deficiency on haemoglobin, haematocrit and non-haem iron in rats

Group	Haemoglobin gm/dL	Haematocrit %	Non-haem iron in liver μg/gm	Non-haem iron in brain μg/gm
Control	15·6±0·6	47·2±1·0	131±8·0	8±0·2
Iron-deficient	15·5±0·4	46·8±1·9	45±1·9*	6·5±0·2†

All values are mean ± sd.
Iron deficient diet: 18–20 mg Fe/kg diet; Control: 250 mg Fe/kg diet.
* $P < 0·001$; † $P < 0·005$.

Table 2. Effect of iron deficiency on ^{3}H-muscimol and ^{3}H-L-glutamate binding to synaptic membranes

Group	% Muscimol binding	% L-Glutamate binding
Control	100	100
Fe-deficient	293.3	37.1
Iron-deficient	Increase by 193·3 %*	Decrease by 62·9 %*

* $P < 0.001$.

63 % in specific binding of ^{3}H-L-glutamate in the iron deficient group as compared to controls (Table 2). The binding could be easily displaced by excess of cold L-glutamate, but not by D-glutamate.

Discussion

The observations on reduction in hepatic and brain non-haem iron in post-weanling rats kept on an iron-deficient diet for two months are in agreement with various reports on latent iron deficiency (Siimes *et al.* 1980; Taneja *et al.* 1986; Taneja *et al.* 1990; Shukla *et al.* 1989*a*).

The significant effects on neurotransmitter receptors during early stages of iron deficiency clearly indicate the deficits in both excitatory and inhibitory pathways of the central nervous system. The neurotransmitter receptors remain in dynamic equilibrium and their regulation depends on the synthesis, metabolism and various other components in the signal transudation cascade (Nakanishi *et al.* 1998). Changes in the affinity of ligands with the receptor can also alter the binding without affecting numbers of receptors present in the system. Fluidity of biological membranes can influence the interaction particularly under *in vivo* conditions (Scheuer *et al.* 1996). Recently, group I metabotropic receptors have been identified which can be modulated by other neurotransmitter receptors including GABA and the ionotropic glutamate receptors (Bordi & Ugolini, 1999). Both GABA and glutamate pathways have been implicated in several nervous system disorders. Dysfunction of the glutamatergic pathway has been suggested in Huntington's disease (Albin *et al.* 1990; Calabresi *et al.* 1999); Alzheimers (Chalmers *et al.* 1990) and epilepsy (Sherwin, 1999). GABA-linked receptor system dysfunction plays an important role in several neurological and psychiatric disorders (Kowell *et al.* 1987). Therefore, it may be logical to suggest that impairment of higher mental functions like cognition and learning in humans may also be linked to changes in neurotransmitter receptors and consequent signal transduction processes in the nervous system.

In an ongoing study in children aged 8–12 years with moderate anaemia or nutritional anaemia or thalassemia, the iron content on globus pallidus, caudate and dentate nuclei was similar in thalassemia as well as in iron deficiency anaemia and in both the anaemic conditions, there was an increase in creatinine and aspartate and reduction in choline concentration (Agarwal, unpublished). These are important alterations as choline is synthesized in the brain in very small amounts; its uptake is Na$^+$ dependent, which requires oxygen.

Conclusions

In latent iron deficiency (without anaemia), brain iron content, neurotransmitters and the related receptors are affected irreversibly during brain development. In contrast, in anaemia the changes are due to anoxia, irrespective of body iron status. Thus in latent iron deficiency iron behaves as a micronutrient inducing specific alterations.

Acknowledgements

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