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EFFECT OF IRON DEFICIENCY ON DEVELOPING RAT BRAIN

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ABSTRACT

Iron deficiency evolves, slowly through several stages. Early iron deficiency caused a depletion in iron stores as shown by a reduction in the levels of hepatic non heme iron (44%) in the 7 days old pups born to iron deficient mothers. The hemoglobin levels and PCV (packed cell volume) were significantly reduced only after the age of three weeks. The activities of GABA (γ-amino butyric acid) shunt enzymes viz. GDH, GAD and GABA-T (glutamate dehydrogenase, glutamate decarboxylase and GABA-transaminase, respectively) and GABA content were found to decrease during gestation and/or lactation iron deficiency at 1 week (wk), 2wk, 3wk and 4wk of age in the developing rat brains. However GABA binding showed a significant increase at different age groups. The iron deficiency during lactational exposure showed significant alteration in most of the parameters only at the age of 3 weeks onwards. On rehabilitation with iron supplemented diet for 2 weeks, these altered parameters could not attain corresponding control values. A prolonged iron deficiency causing alterations in GABA may lead to neurological and behavioral alterations.

KEYWORDS

Iron deficiency, GABA metabolism, Brain - γ -amino butyric acid.

INTRODUCTION

Micronutrient deficiencies are still a major public health problem in many developing countries, with infants and pregnant women especially at risk. Infants warrant extra concern because they require extra micronutrients to maintain optimal growth and development (1). The micronutrient deficiencies which are of greatest public health significance include iron deficiency, which causes varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. low birth weight babies, poor learning capacity and reduced psychomotor skills. Medical evidence show that very severe anaemia is a direct cause of maternal & child mortality (2). In this context the nutritional relation between lactating mothers and their infants is of special interest. Of importance is that the uptake of iron in the brain is at its peak during periods of fast neuronal growth (3). Evidence is strong that in many under developed countries, iron deficiency is main cause of anemia and supplementation under trial condition may prevent some defects of iron deficiency but not the all (4).

Brain is quite sensitive to dietary iron depletion and uses a host of mechanisms to regulate iron flux homostatically. Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is co factor for a number of enzymes involved in neurotransmitter synthesis (5). Further, iron deficiency is associated with alterations in many metabolic processes that may impact brain functioning, among them are-neurotransmitter metabolism, protein synthesis, organogenesis and others (6). Iron deficiency evolves slowly through several stages. In the early stage, referred to as early iron deficiency, the iron stores are depleted without a significant change in hemoglobin levels. However this stage is characterized by iron depletion concomitant with low iron concentration in serum and undersaturation of transferring. It has been proposed that alteration in dopamine, 5HT (Serotonin) receptors that follow iron deficiency, mediate through the neuro developmental changes. Early iron deficiency has also been shown to affect GABA metabolism in adult rats (7, 8).

The purpose of this investigation is to study the effect of maternal iron deficiency by feeding 35mg

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iron/kg diet during pregnancy and/or lactation on development pattern of GABA metabolizing enzymes, its levels and receptor binding in the pups at the age of 1wk, 2wk and 3wk and in the rats of 4wks of age. Further, rehabilitation studies were done to assess the effect of these changes on iron therapy.

MATERIALS and METHODS

Chemicals: The chemicals used were of analytical grade and obtained from E-Merck and SRL. The fine chemicals were obtained from Sigma Chemical Company, St. Louis, MO USA. Radioactive chemicals were purchased from NEN, USA or Amersham International, UK.

Food Materials: Caesin was purchased from Himedia Laboratories Bombay, India and potato starch from SISCO Research Laboratories, India.

Composition of Diet: The iron deficient diet contained casein -210g, starch -640g, ground nut oil -80ml, salt mixture -40g and vitamin mixture - 30g. The composition of salt mixture was according to Hubbel et al (9) with the addition of starch in place of iron citrate, The vitamin composition was according to Williams and Mills (10), where basal diet contained 35mg iron/kg diet and was used as iron deficient diet. For the preparation of iron sufficient diet additional $FeSO_4.7H_2O$ was added as to contain around 390mg iron/kg diet.

ANIMALS AND EXPERIMENTAL DESIGN

Adult male and female wistar albino rats of Industrial Toxicology Research Centre, Lucknow were used in present study. The female albino rats were mated with normal rats. Pregnancy was assumed to have begun by the presence of sperm in vaginal smear The pregnant rats (fed on iron deficient diet for 1wk, 2wk, 3wk and 4wk and control diet) were kept individually in plastic cages covered with stainless steel mesh. Diet and distilled water were given *ad libitum.*

After parturition the average litter weight and number of pups were randomized and litter size was adjusted to eight pups per dam and maintained on either of two diets. The pups were grouped as:

Group A - When the dams were maintained on control diet throughout the gestation – lactation period up to the age of 4 wks.

Group B - When the dams were maintained on iron deficient diet throughout gestation and lactation period up to age of 4 wks

Group C - When the dams were maintained on control diet during gestation but fed on iron deficient diet during lactation period.

Group D - One set, which was fed on iron deficient diet from onset of pregnancy till 2 wks of lactation was transferred to the iron supplemented diet for another 2 wks i.e. till the age of 4 wks. The control group was maintained simultaneously on iron sufficient diet for 4 wks.

Six pups from either sex from each group were sacrificed by decapitation at 1wk, 2wk, 3wk and 4wk of age. Brain and liver of pups were removed. and stored at –20° C for biochemical analysis and preparation of membrane. Freezing of tissue prior to membrane preparation has been shown to have no effect on the receptor activity (11).

All the instruments used during experiments were either of plastic, stainless steel or acid washed glassware to avoid contamination with iron.

ASSAY OF NEUROTRANSMITTER RECEPTORS IN BRAIN PREPARATION

The membranes for GABA receptor binding assay were prepared according to the method of Seth *et al* (11) with some modifications.

The brain tissue was frozen at –20° C for 24 hours and then thawed at room temperature, homogenized and centrifuged at 50,000xg for 14 minutes and final pellet was suspended in cold 40mM Tris buffer, pH 7.4. High affinity binding assays were performed by method of Seth *et al* (11). Binding incubations were carried out in triplicate in a final volume of one ml containing 40mM Tris- HCl buffer, pH 7.4, together with appropriate labelled pharmacological agents.

LKB Wallace Reckbeta II scintillation counter was used for radioactivity measurements. In order to determine the extent of nonspecific binding, control incubations containing unlabelled competing ligands were carried out simultaneously. Binding data is expressed in terms of n moles of [^{3}H] ligand bound/ g protein.

Basic binding characteristics like delineation of saturability, specificity, reversibility and regional distribution was established prior to experiment (11).

ESTIMATION OF GABA LEVELS

Rats were sacrificed by decapitation and brain was removed, weighed and kept at –20°C. The GABA levels were assayed using a HPLC as described by Dravid *et. al.* (12) with some modifications.

ASSAY OF ENZYMES IN BRAIN PREPARATION

The activity of GDH, GAD and GABA – T were determined by the method of Rajlakshmi *et al* (13) with minor modifications. The packed cell volume (PCV) of the blood samples was determined by using standard hematocrit technique (14). The liver nonheme iron and the levels of hemoglobin were estimated in the iron deficient and control groups (15) Protein estimation was done by method of Lowry *et al* (16).

STATISTICAL ANALYSIS

The data collected for various parameters in different groups of dietary treatment was analyzed. Statistical comparisons with the controls were made using the unpaired student's 't' test.

RESULTS

Effect of Maternal Iron Deficiency

Gestation – Lactation Period: There was no significant difference in body weight, brain weight, fur growth and general appearance as well as mortality rates between the offsprings of control and iron deficient mothers. A significant decrease was observed in the levels of hemoglobin, PCV and liver nonheme iron in pups nursing on iron deficient diet (Table 1). The control rats showed an age related increase in the hemoglobin content. The PCV was reduced at 2wk and 3wk of age while liver nonheme iron reduced quite significantly in 7 days old pups.

The metabolizing enzymes of GABA viz; GDH, GAD and GABA-T were significantly reduced at the age of 2wk, 3wk and 4wk while the GABA content was significantly reduced only after the age of 2wk onwards, maximum being at the age of 4 weeks (46%).The activity of MAO in the brain was also found to be significantly reduced at different age groups (Table 1).

The receptor levels of GABA were significantly raised at 2wk and onwards. Scatchard analysis (17) was performed to analyze this alteration in the binding. It was revealed that this increase was due to increase in the maximum number of binding sites (Bmax) without a change in the affinity (K_D) of the receptors (Fig. 1).

Effect of iron deficiency during lactation

There was a decrease in liver nonheme iron (40%) at 3 weeks of age while hemoglobin levels showed no significant reduction in this group. However, the activities of all the GABA metabolizing enzymes were found to be significantly reduced at the age of 3 wks without showing any significant effect at other age groups. The levels of GABA were also found to be reduced significantly only at the age of three wks (Table 1) without showing any significant alteration at other age groups (data not shown). GABA receptor binding was raised at the age of 2 wks (10%) and at 3 wks only by 18% ($p<0.05$), as compared to controls (Fig. 2).

Effect of rehabilitation

When dams were fed on iron deficient diet (35 mg iron/kg diet) from the day one of pregnancy till 2 wks of gestation and then were transferred to the iron supplemented diet for another 2 wks. i.e. 4 wks of age, there was no significant recovery in any of the parameters done except a little recovery in liver iron stores (data not shown).

DISCUSSION

It is evident from the present data that feeding iron deficient diet (35mg iron/kg) during gestation and/ or lactation results in significant decrease in non-heme iron as early as in 7 days old pups showing a stage of early iron deficiency which later entered into the stage of iron deficiency anemia as shown by reduced PCV and hemoglobin levels. This iron deficiency showed no effect on body and brain weights of developing rats. However, there was a significant reduction in the activities of brain GDH, GAD and GABA-T enzymes, associated with GABA metabolism, in gestationally and lactationally iron deficient pups. These decrement in enzyme activities increase with the duration of low iron intake. The brain concentration of GABA was also found to reduce, which may be because of altered metabolism of GABA during iron deficiency anemia in coroboration with the earlier studies (7). Hill *et al*

(19) suggested that there is a great similarity in the brain iron distribution and brain regions that receive inputs from GABA. A decrease in iron content will modulate the GABA release and its metabolism. Further GABA receptor population was found to increase due to an increase in maximum number of binding sites (B_{max}), the reason for this alteration is still unclear. However, earlier studies have reported the involvement of iron in synthesis and packaging of neurotransmitters, their uptake and degradation into other iron containing proteins which may directly or indirectly alter brain function (20). It is likely that lowering of GABA levels during nutritional iron deficiency may be due to the failure to deliver iron to brain during particular period of early brain development. This could be related to delayed motor maturation and perhaps to the behavioral alterations in young humans (21).

A significant reduction in the activity of MAO was also noticed in the developing rat brain. That may be explained in the terms of modulation of dopaminergic neurons because of GABA release. This further suggest that iron status may affect behavior through effects on dopamine metabolism. When experimental rats were rehabilitated with iron sufficient diet for two weeks, hepatic nonheme iron content was slightly recovered without showing any significant recovery in other parameters. This finding may be attributed to slow turnover rate for brain iron compounds (22).

In conclusions this study confirms the earlier reports and further demonstrates that the iron deficiency during developmental stage of brain may cause irreversible disturbances and damage to GABA neurotransmitters system.

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Table 1

Parameters:			1 wk	2 wk	3 wk	4 wk	3 wk (lactation)
(I)	Body Weight (G)	C	9.2 ± 0.2	19.2 ± 0.3	30.4 ± 2.3	42.6 ± 2.1	33.8 ± 1.4
		E	8.9 ± 1.2	18.4 ± 0.6	28.6 ± 2.1	36.6 ± 1.2	30.6 ± 0.6
(II)	Packed Cell volume (%)	C	41.2 ± 2.4	42.6 ± 1.4	39.6 ± 2.5	-	-
		E	38.7 ± 2.8	39.9 ± 2.1*	33.2 ± 2.8*	-	-
(III)	Liver nonheme iron (mg./100g. wet tissue)	C	11.6 ± 1.4	6.1 ± 0.5	7.2 ± 0.4	12.8 ± 1.3	8.2 ± 0 .9
		E	6.5 ± 1.0**	3.8 ± 0.3***	4.9 ± 0.3***	6.6 ± 0.9***	5.6 ± 0.1
(IV)	Hemoglobin Content (g%)	C	7.0 ± 1.8	8.1 ± 1.2	10.0 ± 0.8	11.6 ± 0.7	10.4 ± 0.8
		E	6.4 ± 0.8	6.7 ± 0.6*	7.6 ± 0.6**	7.0 ± 0.6**	9.0 ± 0.7**
(V)	GDH (U/mg of protein)	C	6.9 ± 0.4	8.5 ± 0.6	11.2 ± 0.6	13.4 ± 0.4***	12.1 ± 0.8
		E	5.8 ± 0.6	5.3 ± 0.9*	6.3 ± 0.3***	7.1 ± 0.2	9.8 ± 0.6**
(VI)	GAD (U/g of protein)	C	1.52 ± 0.23	3.12 ± 0.4	4.9 ± 0.5	5.3 ± 0.8	4.1 ± 0.3
		E	0.94 ± 0.54	2.41 ± 0.54**	3.4 ± 0.3***	3.8 ± 0.6***	3.4 ± 0.4**
(VII)	GABA-T U/g of protein	C	4.1 ± 0.36	6.8 ± 0.6	9.7 ± 0.5	12.3 ± 0.4	8.7 ± 0.6
		E	3.8 ± 0.27	5.4 ± 0.5**	7.9 ± 0.6***	9.15 ± 0.4***	7.4 ± 0.39**
(VIII)	GABA levels (mg/g wet weight of brain)	C	1.2 ± 0.4	1.8 ± 0.7	2.6 ± 0.3	3.8 ± 0.75	2.8 ± 0.4
		E	0.9 ± 0.3	0.9 ± 0.4*	1.1 ± 0.87	1.4 ± 0.25	1.9 ± 0.31**
(IX)	MAO (nmoles of benzaldehyde formed min^{-1} mg^{-1} Protein	C	0.85 ± 0.06	1.03 ± 0.13	1.26 ± 0.2	2.8 ± 0.09	1.2 ± 0.6
		E	0.66 ± 0.03*	0.69 ± 0.1*	0.5 ± 0.09*	0.74 ± 0.06***	0.8 ± 0.4***

Table: Effect of feeding iron deficient diet (35mg iron/kg body weight) to rats during gestation &/or lactation period Values are mean ± for six to eight observations. Differs significantly from controls.

**** p < 0.05, ** p < 0.01, *** p < 0.001 considered significant (Student 't'-test)***

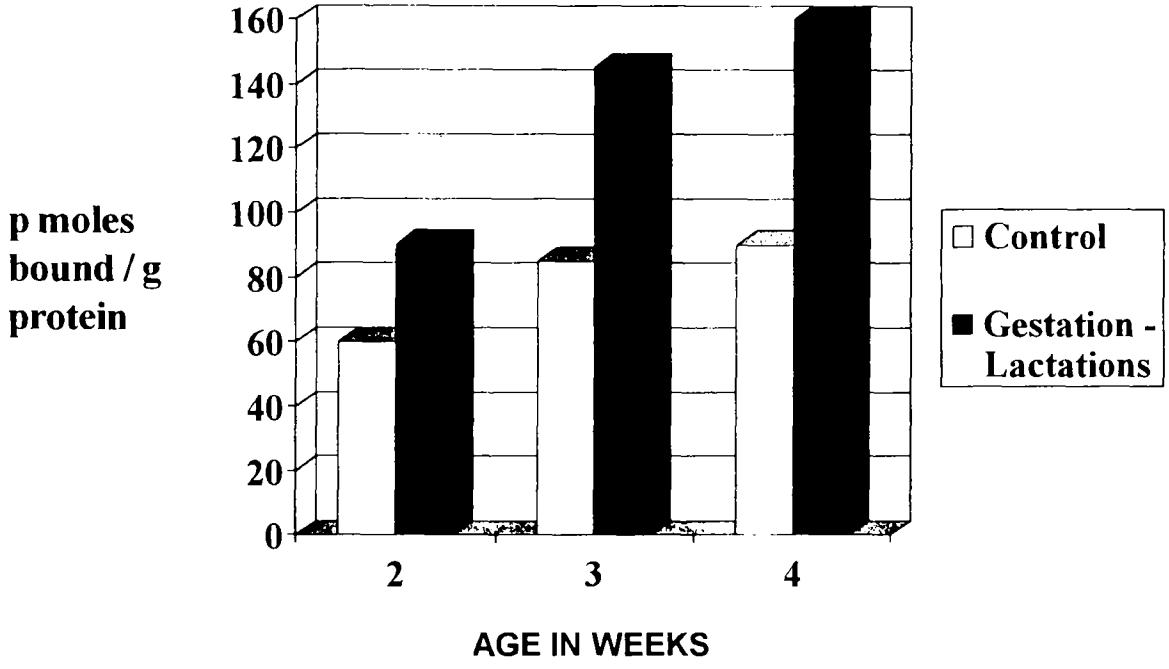

Fig. 1: Alterations induced by gestation - lactation iron deficiency on the [^{3}H] – muscimol receptor levels in brain. Each data is mean + S.E. from six animals of either sex. Differs significantly from controls; * p<0.05, ** p<0.01, ***p<0.001 (student 't' test)

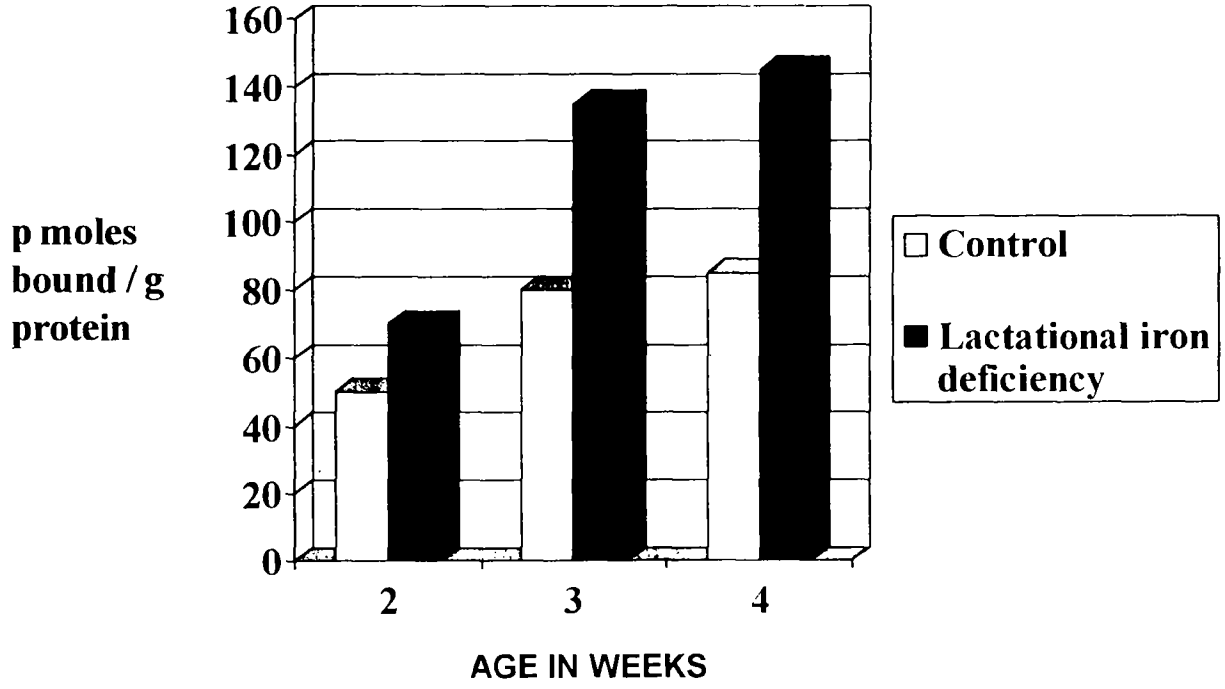

Fig. 2: Alterations induced by lactation iron deficiency on the [^{3}H] – muscimol receptor levels in brain. Each data is mean ± S.E. from six animals of either sex. Differs significantly from controls; * p<0.05, ** p<0.01, ***p<0.001 (student 't'test)