EFFECT OF LATENT IRON DEFICIENCY ON GABA AND GLUTAMATE NEURORECEPTORS IN RAT BRAIN

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ABSTRACT

Eight weeks of latent iron deficiency in weaned female rats of Sprague Dawley strain maintained on experimental low-iron diet (18-20 mg/Kg) did not significantly change the gross body weight and tissue weights of brain and liver. Packed cell volume (PCV) and hemoglobin concentration remained unaltered. However, non-heme iron content in liver and brain decreased significantly ($P < 0.001$). The activities of glutamate dehydrogenase, glutamic acid decarboxylase, and GABA-transaminase (GABA-T) in brain decreased by 15%, 11.4% and 25.7% respectively. However, this decrease was not statistically significant. Binding of ^3H Muscimol at pH 7.5 and 1mg protein/assay increased by 143% ($P < 0.001$) in synaptic vesicular membranes from iron-deficient rats as compared to the controls. ^3H glutamate binding to the synaptic vesicles was also carried out under similar condition. However, the L-glutamate binding was reduced by 63% in the vesicular membranes of iron deficient animals. These studies indicate that iron plays an important functional role in both excitatory and inhibitory neurotransmitter receptors.

KEY WORDS:

Neurotransmitters, Neurotransmitter receptors, ^3H glutamate, [^3H] Muscimol, GABA/glutamate metabolism, brain synaptic vesicles

INTRODUCTION

Iron deficiency is the most important single nutrient deficiency in the world (1). Early stage of iron deficiency known as latent iron deficiency (2) also produces several clinical manifestations. Many of the symptoms are rapidly reversed on iron therapy while others take longer time. The iron deficiency alters a number of biochemical and physiological processes. Of particular importance is the effect on central nervous system (3-4), which leads to the defects in the cognition and learning processes in humans. (5-6) Studies in animal models of latent iron deficiency have shown marked reduction in levels of brain gamma amino butyric acid (GABA) and L-glutamic acid (6-8). Enzymes for biosynthesis of

GABA and L-glutamate like glutamate decarboxylase and glutamate transaminase are also reduced (3,9). These alterations are irreversible particularly in fetal brain because the defect persists even after the rehabilitation. The changes are also specific to iron deficiency because in general protein calorie malnutrition, the effects are reversible. (3,4,10,11)

On the basis of pharmacological and electrophysiological properties, glutamate receptors have been grouped into five subtypes (12-13). These receptors have been implicated in major synaptic network of processing information, coordinating movement pattern and memory (14-15). GABA has two distinct receptors and widely distributed in central nervous system (16-17). The antagonistic function of GABA results from hyper-polarization of pre-synaptic and post-synaptic membrane (18). Both GABA and glutamate play important role in central regulation of physiological processes.

GABA and glutamate neurotransmitters mediate their effects through multiple receptors in the nervous system (19-20). Any changes in the

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neurotransmitters will influence the specific receptors (21). The present study reports the changes in GABA and glutamate receptors in the brain of latent iron deficient rats.

MATERIAL AND METHODS

Female rats of Sprague Dawley strain were used. They were kept in plastic cages with stainless steel mesh. Synthetic diets contained skimmed milk (50%), potato starch (10%) lactose (30%), groundnut oil (5%), salt mixture (4%) and vitamin mixture (1%) (3). Radioligands were purchased from Amersham (UK). All fine chemicals of analytical grade were obtained from Sigma or Merck India.

Iron-deficiency in rats

Female albino rats were used in the experiments. Weaning (21d 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 18-20mg iron/Kg determined by atomic absorption spectrophotometry). For the preparation of iron-sufficient (control) synthetic diet, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added to contain app. 390 mg iron/ Kg diet. Water was served ad libitum in iron-free plastic feeding bottles. At least 6 rats were included in each group.

Both experimental and control groups were fed the diets for 2 months. The rats were sacrificed after anaesthetizing with ether. Blood was collected in plain as well as EDTA vials. Brain and liver tissues were dissected out, rinsed in saline, weighed and frozen at -20°C till the time of processing.

Biochemical and Hematological analysis:

Brain and liver non-heme iron was determined in 10% homogenate according to Hallgreen (22). Glutamate dehydrogenase (L-glutamate NAD oxidoreductase, EC.1.4.1.2), glutamic acid decarboxylase (L-glutamate-1-carboxylase, E.C.4.1.1.15), and gamma- amino-butyric acid transaminase (GABA-T, 4 aminobutyrate: 2-oxoglutarate amino-Transferase, E.C.2.6.1.19) were also assayed in fresh whole tissue homogenate as described earlier (8, 23). Briefly, brain homogenates were prepared in potassium phosphate buffer (0.02M, pH 7.0). The homogenates were incubated in ice for 30 min. after addition of Triton X-100 at a concentration of 2.5mg/ml. The Triton-treated homogenates were used directly for the assay of glutamic acid decarboxylase

and GABA-T activities. The supernatant obtained from centrifugation of above homogenate at $800 \times g$ for 20 min at 4°C was used for glutamate dehydrogenase activity. Glutamate dehydrogenase was assayed in terms of NADH oxidation coupled with reduction of 2-oxoglutarate. For glutamate decarboxylase, GABA formed from L-glutamate was determined after separation with descending chromatography. GABA-T was assayed using 2-oxoglutarate and 4-aminobutyrate as substrates. L-Glutamate formed in the enzymatic reaction was also measured after separation with paper chromatography. Hemoglobin and hematocrit were determined by standard laboratory protocols.

Statistical analysis: Statistical comparisons were made using the Student's *t* test and the results expressed as the mean $\pm$ S.E.

Neurotransmitter receptors:

GABA and L-glutamate receptors in brain were estimated by radio-ligand binding assays in synaptic membranes prepared according to Hell *et al.* (24). For inhibitory neuron-transmitter receptors ^3H -Muscimol, a GABA agonist was used. Binding of ^3H - Muscimol (specific activity 25Ci / mmol) for GABA receptors was done by the method of Seth *et al.* (25). The assay was done in presence or absence of 1×10^{-4} m mol GABA. The incubations were carried out at 37°C for 30 min. At the end of incubation, 2 ml of chilled buffer was added and incubation mixture was immediately filtered through glass fiber filter under vacuum. The filter was rinsed twice in buffer, dried and counted in 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* (26). Binding was carried out in 200 μl volume in micro-titer plates. Optimum pH and protein concentration was determined and found to be 7.4 and 1mg/assay respectively. Binding was carried out at 37°C for 30 min. After incubation, reaction mixture was filtered through Whatman GFB glass fiber filter using a cell harvester as described by Hall and Thor (27). The filters were washed, dried and counted in the liquid scintillation counter.

RESULTS:

Eight weeks of iron deficiency in rat did not significantly change the gross weights of brain and

liver. There was no effect on hemoglobin and hematocrit. The non-heme iron in liver and brain decreased significantly ($P < 0.001$, Table1). The activities of glutamate dehydrogenase, glutamic acid decarboxylase and GABA-T in brain decreased by 15%, 11.4% and 25.7% respectively in iron deficient group. The decrease was, however, not statistically significant (Table2)

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 (1mg / assay). Binding of ^3H - muscimol increased by 193% in membrane from iron-deficient rats as compared to the controls (Table3).

Glutamate Receptors: Like Muscimol, ^3H -glutamate binding was also dependent on pH and concentration of the membrane vesicles. There was significant reduction by 63% in specific binding of ^3H -L glutamate in iron deficient group as compared to the controls (Table3). The binding could be easily displaced by excess of cold L-glutamate, but not by D- glutamate.

DISCUSSION

It is evident from the data that feeding of a marginally iron- deficient diet to the growing rats maintained haemoglobin levels in the normal range for 8 weeks. The observations on reduction in non-heme iron and enzymes in post-weaning rats fed iron deficient diets <35mg iron/kg diet as compared to the control rats for two months are in agreement with various reports on latent iron deficiency (2-4, 8, 28) A significant decrease in the non- heme iron both in liver and brain without changes in hematocrit and hemoglobin suggests appreciable reduction in iron content of certain tissues. Due to absence of anemia during the 8 weeks of iron deficiency, metabolic alterations in rats were rather mild. Li (9) has reported subtle changes in the enzymes and metabolites in similar period of iron deficiency but his animals developed iron deficient anemia.

The significant effects on neurotransmitter receptors during early stages of iron deficiency clearly indicate the deficits in both excitatory and inhibitory pathways of 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 transduction cascade (20). The changes in neurotransmitter receptors may be due to up or down regulation of the receptors. Changes in affinity of ligand with the receptor can also alter the binding without affecting number of receptors present in the system. The mechanism by which iron deficiency changes the binding affinity is unclear. Fluidity of the biological membranes can influence the interaction particularly under *in vivo* conditions (29). The increase in GABA but decrease in glutamate receptor can explain the effects on higher mental functions reported in humans (7, 30). Recently group1 metabotropic receptors have been identified which can be modulated by other neurotransmitter receptors including GABA and the ion tropic glutamate receptors (31). Both GABA and glutamate pathways have been implicated in several nervous system disorders. Dysfunction of glutamatergic pathway has been suggested in Huntington's (32-33), Alzheimer (34) and epilepsy (35). GABA-linked receptor system dysfunction plays important role in several neurological and psychiatric disorders (36). Therefore, it may be logical to suggest that impairment of higher-mental functions like cognition and learning in humans (7) may be linked to changes in neurotransmitter receptors and consequent signal transduction processes in the nervous system. It would be worthwhile to study the effects of iron deficiency on intracellular messengers like Ca, cAMP /cGMP and protein kinases that regulate cellular responses.

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Table 1: Effect of iron deficiency on hemoglobin, hematocrit and non-heme iron in rat (Values given as Mean $\pm$ SE)

Group	Hemoglobin gm/dL	Hematocrit %	Non-heme iron in liver μ g/gm	Non-heme iron in brain μ g/g
Control	15.6 $\pm$ 0.6	47.2 $\pm$ 1.0	131 $\pm$ 8.0	8.0 $\pm$ 0.2
Iron-deficient	15.5 $\pm$ 0.4	46.8 $\pm$ 1.3	45.0 $\pm$ 1.9***	6.5 $\pm$ 0.2**

Iron-deficient diet: 18-20 mg/kg diet; Control: 390 mg Fe/kg diet

***p<0.001; **p<0.005

Table 2: Effect of iron-deficiency on glutamate dehydrogenase (GDH), Glutamic acid decarboxylase (GAD) and GABA transaminase (GABA-T) in rat brain (Values are Mean $\pm$ SE)

Group	GDH ¹ Units/mg protein	GAD ² Units/mg protein	GABA-T ³ Units/mg protein
Control	28.2 $\pm$ 5.2	10.5 $\pm$ 0.2	7.4 $\pm$ 3.7
Iron-deficient	23.0 $\pm$ 5.0*	9.3 $\pm$ 0.9*	5.5 $\pm$ 1.9*

• *not significant

1. n mole of NADH oxidized/min at 30°C
2. n mole of GABA formed at 37° C
3. nmole of glutamic acid formed/min at 37°C

Table 3: Effect of iron-deficiency on ³H-muscimol and ³H-L-Glutamate binding to synaptic membranes

Group	% Muscimol binding	% L-Glutamate Binding
Control	100	100
Iron-deficient	293.3	37.1
% Change	Increase by 193.3%***	Decrease by 62.9%***

*** p<0.001

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