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Nutritional Iron and Dopamine Binding Sites in the Rat Brain

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ASHKENAZI, R., D. BEN-SHACHAR AND M. YOUDIM. *Nutritional iron and dopamine binding sites in the rat brain.* PHARMAC. BIOCHEM. BEHAV. 17: Suppl. 1, 43–47, 1982.—Iron-deficiency (ID) anemia in man is associated with neurological disorders and abnormal behavior. Rats made nutritionally iron-deficient have markedly diminished behavioral responses to centrally-acting drugs (amphetamine and apomorphine) which affect monoaminergic systems. ID has no effect on either the levels of monoamines or on the activities of monoamine-metabolizing enzymes in the brain. We have investigated the possibility that ID may affect postsynaptic events at the level of receptor by measuring the specific binding sites of several neurotransmitters in different brain areas. The results clearly show that ID causes a significant (40–60%) reduction of the DA D_2 binding sites in the caudate. DA-sensitive adenylate cyclase, α- and β-adrenergic, muscarinic cholinergic and the benzodiazepine binding sites were not affected by ID. The effects of ID on DA D_2 binding sites and the behavioral responses to apomorphine can be reversed when iron-deficient rats are placed for 8 days on an iron-deficient diet supplemented with iron. Chronic hemolytic anemia produced by repeated phenylhydrazine injections caused no change in serum iron and had no effect on either apomorphine-induced hyperactivity or ^{3}H-spiroperidol binding in the caudate. Since the highest concentration of iron is found in DA-rich brain areas, it is possible that iron may be crucial to either the synthesis or coupling of the DA D_2 binding site. The possibility that the DA supersensitivity induced by neuroleptics may be related to iron metabolism in the brain has been investigated.

Nutritional iron Dopamine binding sites

IRON is the most abundant trace metal in the body, and in the brain its distribution parallels that of dopamine (DA). The highest concentrations are found in the globus pallidus, caudate nucleus, putamen and substantia nigra and the lowest in the cortex and cerebellum [18,43]. The excess accumulation of iron in the brain has been associated with a number of neurological disorders. Thus, the increased brain iron in the globus pallidus reported in Hallervorden-Spatz disease [12, 27, 36] has been associated with disturbances in gait. A histochemically demonstrable increase of iron in the cortex of subjects with Alzheimer's disease has been reported [16]. Increased brain iron has also been shown in cases of Huntington Chorea [8,36], as well as Kaschin-Beck's disease [19]. Of interest is the increased amounts of iron found in the globus pallidus and the caudate nucleus of psychotic and schizophrenic patients [37,38]. This has been attributed to a slower turnover of brain iron compared to controls. On the other hand, iron-deficiency is a prevalent nutritional disorder [14,22] and is also thought to affect behavior in children and adults [28, 29, 30] and in the rat [15,43].

It has been shown [43] that the serotonin (5-HT) and the DA mediated behavioral responses induced by centrally-acting drugs were significantly diminished in iron-deficient rats. The DA mediated hypothermia induced by d-amphetamine in rats kept at 4°C was also decreased in iron-deficient rats [45]. The altered behavioral response could not be attributed to changes in monoamine metabolism, since the activities of the enzymes tryptophan hydroxylase, tyrosine hydroxylase and monoamine oxidase were the same in control and iron-deficient rats. Furthermore, no changes in the turnover and brain levels of 5-HT, DA or noradrenaline (NA) were observed [44]. It was suggested that the reduction in the functional activity of monoaminergic pathways induced by nutritional iron-deficiency might be due to postsynaptic changes at or beyond the DA receptor site [44]. Supporting evidence for this has come from the similarity of the effects of iron-deficiency on the DA-mediated behavioral and thermo-regulatory responses, and the effects of neuroleptics on these responses [17, 41, 42]. We have investigated the effect of nutritional iron-deficiency on the DA binding sites and on the neuroleptic-induced supersensitivity of these binding sites in the rat brain, and correlated them with behavioral changes.

METHOD

Sprague-Dawley rats (21 days old) were fed a semi-synthetic diet low in iron [26] and distilled water. Control rats were given the same diet supplemented with ammonium ferrous sulphate (1.3 g/kg diet). The iron-deficient rats were given an ad lib diet. However, the control group had their food intake restricted to that of the iron-deficient group in order to keep their body weights similar. In order to assess the iron-deficiency state of the animals, each week tail blood was taken for the determination of hemoglobin. Iron repletion was achieved by placing the iron-deficient rats on the control diet for 14 days. Hemolytic anemia was produced in control rats by a daily intraperitoneal injection of 50 mg/kg

TABLE 1

EFFECT OF IRON-DEFICIENT DIET ON HEMOGLOBIN, SERUM IRON AND APOMORPHINE-INDUCED MOTOR ACTIVITY

Diet	Hemoglobin (g/100 ml)	Serum Iron (μg/ml)	Apomorphine-induced motor activity movement/40 min
Control	14.1 ± 1.2 (12)	3.3 ± 0.3 (6)	1614 ± 72 (4)
Iron deficient	5.9 ± 0.6* (12)	0.76 ± 0.07* (6)	798 ± 159* (4)
Iron repleted† (control diet)	13.6 ± 2.1 (8)	3.35 ± 0.3 (9)	1462 ± 300 (4)

The results are expressed as means±S.E.M.
*Significantly different from control and iron-repleted groups ($p<0.01$).
†Iron-deficient animals were placed on control (iron-supplemented) diet for 2 weeks.

TABLE 2

EFFECTS OF DIET ON DOPAMINE BINDING SITES

Diet	Adenylate cyclase activity pmol/2 mg tissue/2.5 min* Basal	Adenylate cyclase activity pmol/2 mg tissue/2.5 min* DA-stimulated	^{3}H-spiroperidol binding Kd (nM)	^{3}H-spiroperidol binding B(max) fmole/mg protein
Control	68.0 ± 1.4 (6)	136 ± 20.4 (6)	0.72 ± 0.2 (4)	306 ± 60 (4)
Iron-deficient	62.6 ± 2.4 (6)	131 ± 13.1 (6)	0.68 ± 0.1 (4)	128 ± 13† (4)
Iron-repleted (control diet)	—	—	0.81 ± 0.2 (4)	243 ± 40‡ (4)

The results are expressed as means±S.E.M.
*Values taken from the studies of Youdim *et al.* [43].
†Significantly different from control and iron-repleted groups ($p<0.01$).
‡Not significantly different from control.

phenylhydrazine for one week, followed by three weeks of a daily injection of 30 mg/kg phenylhydrazine. DA supersensitivity was produced in control and iron-deficient rats by the daily administration of haloperidol (10 mg/kg sc). Haloperidol injections started on the first day of the diet and continued for 4 weeks. In these animals behavioral and binding studies were performed 48–72 hours after the last injection of haloperidol.

Locomotor activity was monitored in groups of three animals in cages placed on a Varimax activity meter (Colombus Instruments). Hemoglobin was measured by modification of a method described [13] using the commercial kit from Sigma (U.S.A.). Serum iron was determined by assay [5]. 5-HT was assayed fluorometrically [10] and DA and NA were also determined [6]. DA-sensitive adenylate cyclase was assayed [20] and cyclic AMP was measured [4].

Specific ^{3}H-spiroperidol binding was measured *in vitro* in membranes prepared from the caudate nucleus [9]. Specific binding was defined as the difference between the total counts in the absence of cold haloperidol and the counts obtained in the presence of haloperidol (10 μM).

Protein was also determined [24].

TABLE 3

EFFECTS OF PHENYLHYDRAZINE TREATMENT

	Control	Phenylhydrazine
Hemoglobin (g/100 ml)	12.7 ± 1.2 (5)	7.1 ± 0.5* (5)
Serum iron (μg/ml)	3.1 ± 0.9 (5)	4.7 ± 0.7 (5)
Specific ^{3}H-spiroperidol binding at 1 nM (pmoles/g tissue)	20.7 ± 3.0 (5)	22.6 ± 3.0 (5)
Apomorphine-induced motor activity (movements/40 min)	1065 (2)	1028 (2)

The results are expressed as ±S.E.M. with number of observations in brackets.
*Significantly different from control (Student's t-test), $p<0.01$.

TABLE 4

EFFECT OF CHRONIC HALOPERIDOL TREATMENT IN CONTROL AND IRON-DEFICIENT RATS

Treatment	^{3}H-spiroperidol binding (fmole/mg protein)	Hemoglobin (g/100 ml)	Serum iron (μg/ml)
Control	207 ± 31 (8)	14.5 ± 1.1 (8)	2.38 ± 0.4 (8)
Control + haloperidol	256 ± 31* (7)	14.6 ± 1.4 (8)	2.76 ± 0.45 (8)
Iron-deficient	160 ± 28 (8)	7.8 ± 2.8* (8)	1.04 ± 0.1* (8)
Iron-deficient + haloperidol	239 ± 46† (7)	6.5 ± 1.5* (7)	0.97 ± 0.18* (7)

Results show mean±S.D. with number of observations in bracket.
*Different from control (Student t-test) $p<0.01$.
†Different from iron-deficient (Student t-test) $p<0.005$, but not different from the control.

RESULTS

Iron Deficiency and Dopamine Binding Sites

It has been shown [43] that in iron-deficient rats, whose brain iron stores were significantly lowered, the activities of iron-dependent monoamine enzymes, tryptophan hydroxylase, tyrosine hydroxylase and monoamine oxidase, were unchanged. However, the behavioral response of these rats to amphetamine and apomorphine was significantly diminished without a change in the basal level or turnover of 5-HT, NA and DA. In this study we have confirmed the observation that the locomotor response to apomophine administration is decreased in iron-deficient rats. After four weeks on a diet low in iron, there was a significant decrease, not only in the hemoglobin level and in the concentration of serum iron, but also in the motor activity induced by apomorphine (2 mg/kg IP) (Table 1). The possibility that the diminished behavioral response to apomorphine could be due to postsynaptic changes at the level of the DA receptor was investigated by monitoring the activity of the DA-sensitive adenylate cyclase and by examining the specific binding of ^{3}H-spiroperidol in the caudate nucleus (Table 2). The basal activity, as well as the *in vitro* stimulation of the DA-sensitive adenylate cyclase in the caudate nucleus, were the same in control and iron-deficient rats. However, a significant decrease in the specific ^{3}H-spiroperidol was observed in iron-deficient rats. Scatchard analysis of the saturation curves of ^{3}H-spiroperidol binding showed that iron-deficiency reduced the maximum number of binding sites (Bmax) by 60% but had no effect on the dissociation constant (K_D).

When the iron-deficient rats were given the iron-plus (control) diet for two weeks, there was complete restoration, not only of the hemoglobin concentration, the serum iron level and the behavioral response to apomorphine (Table 1), but also of the Bmax for ^{3}H-spiroperidol binding (Table 2).

Effects of Hemolytic Anemia on Behavior and ^{3}H-Spiroperidol Binding

To evaluate the role of anemia *per se* on the specific binding of ^{3}H-spiroperidol, hemolytic anemia was induced by chronic phenylhydrazine administration. Although phenylhydrazine-treated rats had significantly lower hemoglobin levels compared with control rats, there was no change either in their serum iron levels nor in ^{3}H-spiroperidol binding sites in the caudate nucleus (Table 3). Furthermore, their behavioral response to apomorphine (2 mg/kg) was similar to control rats.

Effect of Chronic Haloperidol Treatment on ^{3}H-Spiroperidol Binding in Control and Iron-Deficient Rats

The effects of chronic haloperidol administration (10 mg/kg SC for 4 weeks) on ^{3}H-spiroperidol binding and behavior were studied in rats fed either the control or the iron-deficient diet. Haloperidol treatment had no effect on either hemoglobin levels or serum iron concentrations in either group (Table 4).

As expected, iron-deficiency decreased the behavioral response to apomorphine and ^{3}H-spiroperidol binding in the caudate nucleus, while chronic treatment of control rats with haloperidol increased these parameters (Table 4). However, in iron-deficient rats, chronic haloperidol administration prevented the diminution of apomorphine-induced behavior, as well as ^{3}H-spiroperidol binding. The values for the latter were not significantly different from control rats treated with haloperidol.

DISCUSSION

The distribution of non-hem iron in the brain of rats [44] and humans [18] parallels that of DA. In some brain regions, such as the caudate nucleus, globus pallidum and hypothalamus, the non-hem iron concentration is as high as that of the liver, the main site of iron storage and metabolism. The major portion of non-hem iron is associated with the crude mitochondrial-synaptosomal fraction and myelin [7, 32, 43]. However, the functional role of brain iron is not known. Rats made iron-deficient have significantly lower brain non-hem iron and behave differently to treatment with apomorphine and amphetamine and other centrally-acting drugs when compared to control rats [11, 25, 43]. Induction of iron-deficiency results not only in the diminution of certain behaviors mediated by central dopaminergic and adrenergic neurons, but also in the reversal of their daily rhythm. These altered behavioral changes are not related to a modification in the metabolism of DA or NA or 5-HT, since the turnover and brain levels of these amines, as well as the brain activities of the iron-dependent enzymes tyrosine hydroxylase, tryptophan hydroxylase and monoamine oxidase, are unaffected by a nutritional deficiency of iron [31, 43, 44]. The behavioral and biochemical studies of Youdim and Green

indicated that alteration in 5-HT- and DA-mediated behavior might be due to postsynaptic changes. The cerebral DA binding sites have been classified into two groups, one type being linked to adenylate cyclase (D_1 receptor) and the other unrelated to the activation of cyclic nucleotides (D_2 receptor) [21,35]. Apomorphine acts on both types of dopamine receptors [21]. It has been reported [44] that neither the basal activity of caudate nucleus DA-sensitive adenylate cyclase, nor its response to increasing concentrations of DA, were affected by iron-deficiency. However, in this study we found that specific ^{3}H-spiroperidol binding, which has been used in the estimation of the D_2 binding site, was markedly reduced in iron-deficient rats exhibiting reduced behavioral responses to apomorphine. The decrease in the specific binding was due to a decrease in the number of binding sites in the iron-deficient rats. This effect of iron-deficiency seems to be specific to the D_2 binding sites, as neither α- nor β-adrenergic binding sites, nor cholinergic muscarinic binding sites, were affected by iron-deficiency (unpublished observation).

Further support that DA D_2 binding sites are decreased in other brain regions (e.g., pituitary) has come from our studies on the regulation of liver prolactin receptor [3]. Chronic treatment of rats with neuroleptics (chlorpromazine, haloperidol or fluphenazine), which increase plasma prolactin, causes a significant (200–300%) induction of prolactin receptor in the liver [2]. Similar increases are also noted in the livers of iron-deficient rats [2]. This regulation of liver prolactin receptors by neuroleptics and iron-deficiency is thought to be mediated by prolactin itself, since chronic treatment of rats with low doses of prolactin (0.1 μg/kg for 7 days) brings about the same effect [1].

The diminution in the D_2 binding sites is not due to anemia *per se*, since hemolytic anemia produced by phenylhydrazine caused a marked decrease in hemoglobin without a change in either serum iron concentration or in the specific binding of ^{3}H-spiroperidol in the caudate nucleus or in the behavioral response to apomorphine. The effect of iron-deficiency on the D_2 site is reversible. When the iron-deficient rats were given the control diet for 2 weeks, there was a complete correction of the anemia, and both the behavioral response to apomorphine and the ^{3}H-spiroperidol binding returned to control levels.

The mechanism by which iron-deficiency has a selective action on dopamine binding in the caudate nucleus is not known. It may be the result of one or more of the following possibilities: (a) iron could be part of the receptor and may be required for the binding of dopamine or its antagonists; (b) the attachment of the postsynaptic receptor to the neuronal membrane is mediated by iron; and (c) synthesis of the D_2 dopamine binding sites is dependent on an adequate supply of iron. In the latter case it is known that iron-deficiency results in a significant reduction of protein synthesis in the brain [40], and this may be the reason for the slow rate of reappearance of DA binding sites after iron-deficient rats have been placed on an iron-plus diet.

It is well documented that D_2 receptors increase in density in the striatum after long-term administration of various neuroleptics [34]. However, iron-deficiency failed to abolish the increase in ^{3}H-spiroperidol-binding produced by chronic treatment with haloperidol. One possibility is that haloperidol and other neuroleptics may affect iron turnover in the brain. It is important to note that chronic administration of chlorpromazine, which causes dopamine supersensitivity, increased iron concentrations in the caudate nucleus of the guinea pig [39]. If haloperidol has the same effect as chlorpromazine, then this excess of iron in the brain may prevent the decrease in D_2 binding sites caused by iron deficiency. Thus, neuroleptics may have the property of either mobilizing iron stores from the periphery to the CNS or preventing its efflux from the brain due to their strong iron-chelating property [33].

The present results indicate that the altered behavioral responses of iron-deficient rats to centrally-acting drugs are due to a change in the DA D_2 binding site in the brain and not to a peripheral phenomenon, as was suggested [23]. This CNS effect seems to occur independently of anemia. From these observations and the reported increase in brain iron-levels in the neurological disorders discussed in the introduction, it is apparent that the modulation of iron in the brain can bring about a change in dopaminergic activity. The possibility exists that neuroleptic-induced dopamine supersensitivity and tardive dyskinesia may be associated with a lower turnover rate of iron in the brain, resulting in an increased level of this trace metal. This hypothesis is partially supported by our finding that haloperidol treatment in iron-deficient rats prevented the decrease in DA D_2 binding sites.

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