Iron deficiency and neurotransmitter synthesis and function

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There has been little work on the effects of iron-deficiency on brain metabolism and function despite the fact that Fe deficiency is the most common nutritional disorder in the world (Garby, 1973; Kossner & Kalk, 1973).

Fe is present in the brain in relatively large amounts and is particularly concentrated in the basal ganglia (Cumings, 1948, 1968; Hallgren & Sourander, 1958; Harrison, Netsky & Brown, 1968). Furthermore it's concentration appears to be independent of the liver Fe stores (Hallgren & Sourander, 1958; Dallman, 1974).

It has been suggested that Fe is a cofactor for tyrosine hydroxylase (*EC* 1.14.16.2) (Sourkes, 1972) and tryptophan hydroxylase (*EC* 1.13.11.11) (Ichiyama, Nakamura, Nishizuka & Hayashi, 1968; Lovenberg, Jequier & Sjoerdsma, 1968; Youdim, Hamon & Bourgoin, 1975), enzymes involved in the formation of catecholamines and 5-hydroxytryptamine (5-HT) respectively. The activity of the monoamine degradative enzyme monoamine oxidase (*EC* 1.4.3.4; MAO) has also been shown to be lowered in the tissues of both humans and rats with Fe-deficiency anaemia (Symes, Sourkes, Youdim, Birnbaum & Gregoriadis, 1969; Voorhess, Stuart, Stockman & Oski, 1975; Youdim, Woods, Mitchell, Grahame-Smith & Callender, 1975; Youdim, Grahame-Smith & Woods, 1976).

We have now investigated the effects of Fe deficiency in rats on the activity of various monoamine-metabolizing enzymes in the brain. The functional activity of the monoamine neurotransmitter systems has also been investigated by measuring the behavioural responses of the rats to increased brain 5-HT and dopamine (DA) receptor stimulation.

Preparation of Fe-deficient rats

Male Sprague-Dawley rats (Anglia Laboratory Animals, Huntingdon), initial weight 80 ± 20 g (mean$\pm$SEM) were made Fe deficient by feeding on a semi-synthetic diet of milk powder low in Fe (McCall, Newman, O'Brien, Valberg & Witts, 1962) and distilled water. Control rats were given tap-water and the milk-powder diet with ammonium ferrous sulphate ($1 \cdot 3$ mg/g diet) added. The Fe-deficient group were given *ad lib.* access to the diet while the control group had their food intake restricted to that of the Fe-deficient group. After 5 weeks the

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animals were judged to be Fe deficient (haemoglobin (g/l): control group $141 \pm 4 \cdot 4$ (*n* 16), Fe-deficient group $55 \cdot 5 \pm 4 \cdot 2$ (*n* 22)).

Distribution of non-haem-Fe in the brain

The regional distribution of non-haem-Fe is reported in Table 1 and in agreement with Dallman, Simes & Manies (1975) a mean brain concentration of $0 \cdot 074$ µg non-haem-Fe/mg protein was found. The Fe-deficient rats had a mean non-haem-Fe concentration of $0 \cdot 030$ µg/mg protein, showing a 60% reduction. The distribution (%) between the subcellular functions was unchanged in Fe deficiency showing that there are no specific effects on this distribution.

Table 1. *Distribution of non-haem-iron in various areas of rat brain*

(The results shown are mean values with their standard errors for six animals)

	Fe (µg/mg protein)	
Brain areas	Mean	SE
Cerebellum	0·075	0·008
Brain stem	0·052	0·008
Caudate nucleus	0·092	0·036
Amygdala	0·058	0·005
Cerebral cortex	0·050	0·003
Hypothalamus	0·076	0·030

Monoamine-metabolizing enzymes during Fe deficiency

In rats with haemoglobin values of 69 ± 6 g/l no decrease in tryptophan hydroxylase activity was found; nor was brain MAO activity lowered even though there was a large decrease in the activity of this enzyme in the liver, heart and adrenal gland (though not the spleen) (Fig. 1).
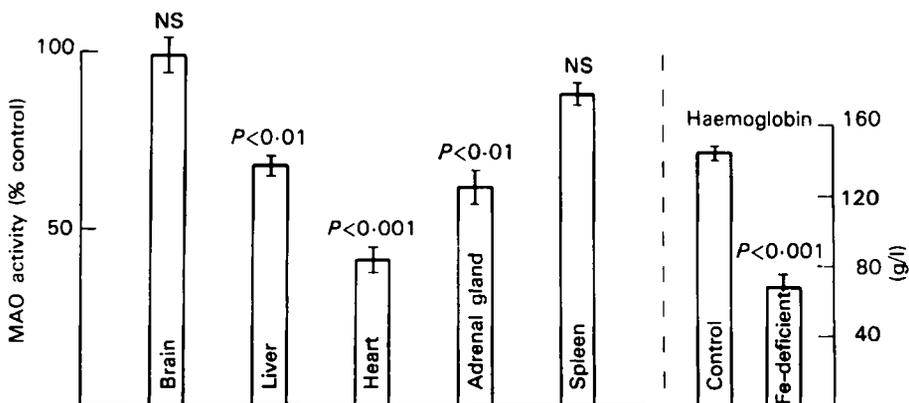

Fig. 1. The effect of iron-deficiency on rat tissue monoamine oxidase (*EC* 1.4.3.4) activity. The activity was measured in tissue homogenates with kynuramine as substrate: NS, not significant. Enzyme activity was measured in tissues of animals which had been fed on an Fe-deficient or Fe-adequate (control) diet for at least 5 weeks.

Brain, liver and heart aldehyde dehydrogenase (EC 1.2.1.3) was also unaltered in these animals. In a group of rats made severely Fe deficient by keeping them on the Fe-deficient diet for 9 weeks (haemoglobin 45 ± 6 g/l) there was still no decrease in tryptophan hydroxylase activity, although brain MAO showed a small but statistically significant decrease in activity (15%). The flavoprotein-containing succinate dehydrogenase (EC 1.3.99.1; SDH) also showed little change in the brain of these animals although the activity in peripheral tissues was markedly decreased.

The activity of brain enzymes which may be Fe dependant appear therefore to be very resistant to the effects of Fe deficiency. Fe is therefore either still present in sufficient quantities to act as a cofactor, or the enzymes do not require Fe (which seems to be unlikely in view of the big changes seen in the peripheral activity of MAO and SDH) or is bound in some form which simple nutritional deficiency will not alter.

Effects of Fe deficiency on the functional activity of brain 5-HT and DA

Administration of a MAO inhibitor (e.g. tranylcypromine) followed by L-tryptophan results in rats displaying various behavioural changes, one feature of which is hyper-reactivity, which can be measured on Animex activity meters (Grahame-Smith, 1971a). Previous studies have suggested that the extent of hyperactivity is proportional to the rate of increase in 5-HT synthesis and 'spill-over' into functional activity in the brain (see Green & Grahame-Smith, 1976). A similar behavioural model can be elicited by an injection of tranylcypromine and L-dopa, a procedure which increases brain DA concentration and this activity has been used as an index of brain DA functional activity (Green & Kelly, 1976). By using these behavioural models together with measurement of activity after injection of the putative 5-HT agonist 5-methoxy-N,N-dimethyl-tryptamine (5-MeODMT) or the DA-releasing drug amphetamine or DA agonist apomorphine, changes in both pre- and post-synaptic monoamine function can be assessed.

After tranylcypromine–L-tryptophan administration, Fe-deficient rats show much less response than control animals (Fig. 2). However, the rate of brain 5-HT accumulation and 5-HT synthesis was the same in both groups (Table 2) supporting the finding that tryptophan hydroxylase activity is unchanged. Endogenous brain 5-HT correlations were decreased in Fe deficiency perhaps due to decreased 5-HT binding and storage at the nerve ending, since Fe has been shown to be involved in this process (Tamir, Klein & Rapport, 1976). The decreased activity appears to be due to post-synaptic changes since the response to 5-MeODMT was also inhibited (Table 3).

There were also alterations in DA function, the behavioural responses to tranylcypromine–dopa, methamphetamine and apomorphine being inhibited (Table 3) although again there was no alterations in DA accumulation (Table 4). The biochemical evidence and the changed apomorphine response again point to a post-synaptic change. We were not however, able to detect any alteration in DA-sensitive adenylate cyclase (EC 4.6.1.1) activity.
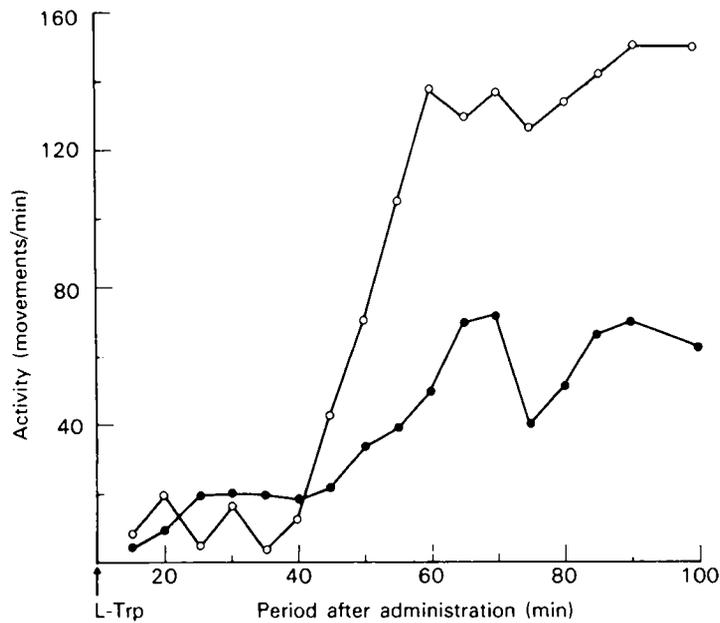
Fig. 2. The behavioural effect (activity; movements/min) of L-tryptophan (L-Trp) given after administration of tranylcypromine (monoamine oxidase (*EC* 1.4.3.4) inhibitor). Rats were injected with tranylcypromine (20 mg/kg) and L-Trp (100 mg/kg) was given 30 min later. (○—○), Control; (●—●), Fe-deficient. Statistical evaluation is given in Table 3.

Table 2. *The effect of iron deficiency on the synthesis of rat brain 5-hydroxytryptamine (5-HT)*

(The results shown are mean values with their standard errors for no. of observations given in parentheses; results are expressed as μg/g brain wet weight. The rate of 5-HT synthesis was determined by the accumulation of 5-HT in excess of the control value during 60 min after injection of tranylcypromine (a monoamine oxidase (*EC* 1.4.3.4) inhibitor). Haemoglobin concentrations in these rats are given in Table 4. 5-HT accumulation was measured 60 min after injection of L-tryptophan (100 mg/kg) with tranylcypromine (20 mg/kg) being given 30 min before the tryptophan)

	Control		Fe-deficient	
	Mean	SE	Mean	SE
Brain tryptophan (μg/g)	3·33	0·26 (4)	3·69	0·13 (5)
Brain 5-HT (μg 5-HT/g)	0·38	0·02 (6)	0·31	0·02 (9)●
Brain 5-HT 60 min after tranylcypromine (μg 5-HT/g)	0·51	0·02 (4)	0·42	0·01 (5)●
Rate of 5-HT synthesis (μg 5-HT/g per h)	0·13		0·11	
5-HT accumulation after tranylcypromine–L-tryptophan (μg 5-HT/g)	0·095	0·07 (11)	0·91	0·06 (7)

●Different from control values: *P* < 0·01.

Whether these changes are occurring because of a change in DA receptor function (although perhaps not the adenylate cyclase system) or the function of another neuronal system modulating DA function cannot, at present, be stated.

When the Fe-deficient rats were given the Fe diet for 8 d their responses to tranylcypromine–L-tryptophan and tranylcypromine–L-dopa returned to normal. The haemoglobin value had also increased ($122\pm6\cdot4$ g/l).

We also confirmed the finding of Glover & Jacobs (1972), that there is an altered pattern of spontaneous activity in Fe-deficient rats, confirming that they are less active during the dark period (Table 3).

Table 3. *The effect of iron deficiency on the behavioural response (activity; no. of movements) of rats to various drugs*

(The results shown are mean values with their standard errors for no. of observations given in parentheses. Activity was measured on groups of three rats with an Animex activity meter sensitivity and tuning: 30 µA) as described elsewhere (Grahame-Smith, 1971)).

	Control		Fe-deficient	
	Mean	SE	Mean	SE
During 90 min after L-Trp	4300	661 (4)	1682	304 (4)●
During 60 min after L-dopa	4576	671 (4)	1971	786 (3)●●
During 40 min after 5-MeODMT	2885	240 (3)	1335	410 (3)●●●
During 90 min after methamphetamine	8977	1145 (4)	5093	1188 (3)●●●
During 40 min after apomorphine	3282	385 (4)	1488	642 (3)●●●
Activity during overnight period (18.00 hours–10.00 hours)	33690	2791 (3)	19395	2230 ●

L-Trp, L-tryptophan; 5-MeODMT, 5-methoxy-N,N-dimethyltryptamine.
Different from control values: ●$P<0\cdot01$, ●●$P<0\cdot02$, ●●●$P<0\cdot05$.

Table 4. *The effect of iron deficiency on rat brain catecholamine concentrations (µg/g brain wet weight) and on haemoglobin (g/l)*

(The results shown are mean values with their standard errors for no. of observations in parentheses. Accumulation of dopamine and noradrenaline was measured 60 min after injection of L-dopa (50 mg/kg), tranylcypromine (20 mg/kg) having been given 30 min before the L-dopa).

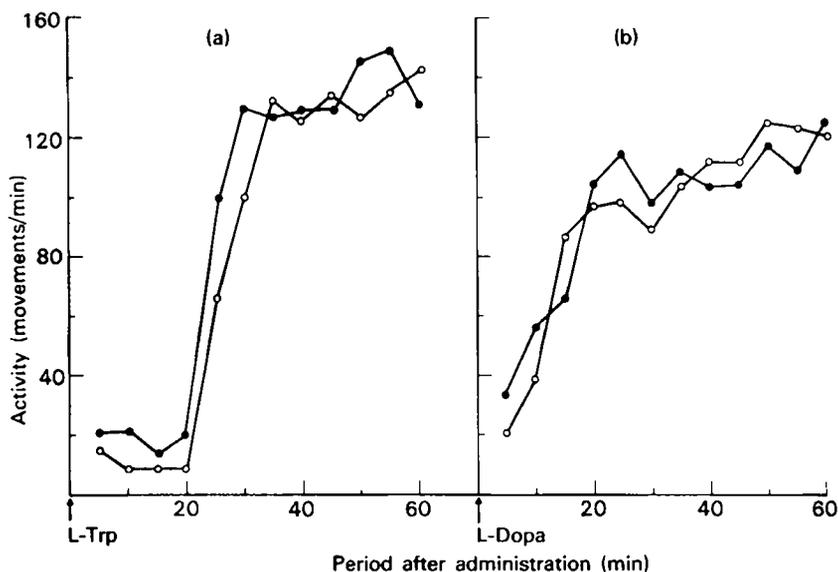|	Control		Fe-deficient	
	Mean	SE	Mean	SE
Brain dopamine (µg/g)	2·03	0·24 (6)	1·74	0·13 (8)
Brain noradrenaline (µg/g)	0·19	0·01 (4)	0·20	0·01 (5)
Brain dopamine after tranylcypromine–L-dopa	5·72	0·32 (5)	5·75	0·57 (6)
Brain noradrenaline after tranylcypromine–L-dopa	0·54	0·76 (6)	0·55	0·04 (6)
Haemoglobin	141·0	4·4 (16)	55·5	4·2 (22)●

●Different from control values: $P<0\cdot001$.

Fig. 3. The behavioural effect (activity; movements/min) of injecting (a) L-tryptophan (LTrp) or (b) L-dopa after a dose of tranylcypromine (monoamine oxidase (*EC* 1.4.3.4) inhibitor) on hyperactivity in iron-deficient rats given an Fe-adequate diet. Fe-deficient rats (haemoglobin 69 ± 5 g/l) were placed on Fe-adequate diet for 8 d and then injected with tranylcypromine (20 mg/kg) and, 30 min later, either L-Trp (100 mg/kg) or L-dopa (50 mg/kg). (O—O), Control; (●—●), Fe-deficient.

Conclusions

We cannot exclude the possibility that the effects of the anaemia or changes in muscle activity are responsible for the changes seen. They are, however, unlikely for several reasons. If the lowered activity were due to an oxygen deficit because of the anaemia, then the activity would have decreased as the experiment progressed. However the lowered responses continue for more than 2 h after tryptophan injection and increase with the period of time after injection. Dallman (1974) has reported that Fe-deficiency does not alter isolated muscle function and Edgerton, Bryant & Gillespie (1972) did not demonstrate a relationship between physical performance and muscle content of myoglobin and cytochromes.

The neurotransmitters 5-HT, DA and noradrenaline are intimately involved in the regulation of mood and neuronal activity. In view of the prevalence of Fe deficiency in the world, particularly young children (Pollitt & Leibel, 1976) we suggest the long-term effects of Fe deficiency on brain neuronal systems should be further investigated.

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Printed in Great Britain