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Review Article

Altered Brain iron homeostasis and dopaminergic function in Restless Legs Syndrome (Willis–Ekbom Disease)

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ABSTRACT

Restless legs syndrome (RLS), also known as Willis–Ekbom Disease (WED), is a sensorimotor disorder for which the exact pathophysiology remains unclear. Brain iron insufficiency and altered dopaminergic function appear to play important roles in the etiology of the disorder. This concept is based partly on extensive research studies using cerebrospinal fluid (CSF), autopsy material, and brain imaging indicating reduced regional brain iron and on the clinical efficacy of dopamine receptor agonists for alleviating RLS symptoms. Finding causal relations, linking low brain iron to altered dopaminergic function in RLS, has required however the use of animal models. These models have provided insights into how alterations in brain iron homeostasis and dopaminergic system may be involved in RLS. The results of animal models of RLS and biochemical, postmortem, and imaging studies in patients with the disease suggest that disruptions in brain iron trafficking lead to disturbances in striatal dopamine neurotransmission for at least some patients with RLS. This review examines the data supporting an iron deficiency–dopamine metabolic theory of RLS by relating the results from animal model investigations of the influence of brain iron deficiency on dopaminergic systems to data from clinical studies in patients with RLS.

1. Introduction

Restless legs syndrome (RLS), also known as Willis–Ekbom disease (WED), is a common sensorimotor disorder that has a prominent circadian pattern [1]. Evidence has accumulated for the role of brain iron insufficiency and dopamine (DA) neurotransmission abnormalities in the etiology of RLS. The suspected role of a DA abnormality in RLS is due in large part to the remarkable treatment response seen with levodopa and dopaminergic (DAergic) agonists in alleviating RLS symptoms versus the symptom exacerbation observed with DA antagonists [2–6]. However, the mechanisms by which abnormalities in DAergic neurotransmission or in DA metabolism result in the development of RLS have remained elusive.

An association between iron deficiency and RLS was originally identified by Nordlander in the 1950s [7]. Further studies have shown a higher prevalence of RLS symptoms in conditions that compromise iron availability [8]. A recent study in a population of patients with iron-deficiency anemia reported finding a 31.5% prevalence of RLS [9], which is six times higher than the general USA population prevalence for RLS [10]. Most patients with RLS, however, do not have an obvious iron deficiency. This point was evident to Nordlander, who proposed that: "It is possible, however, that there can exist an iron deficiency in the tissue in spite of normal serum iron." [11] True to that hypothesis, despite the lack of an apparent systemic iron deficiency in most RLS patients, an iron-insufficient state appears to exist in the brains of RLS patients [2]. This brain-specific deficit in iron may be a consequence of the tight regulation of iron transportation by the blood–brain barrier [12]. Iron is normally transported into the brain from the plasma by the choroid plexus within the cerebral ventricles and by binding to the transferrin receptor expressed on endothelial cells in the brain microvasculature [13–15]. However, recent postmortem studies by Connor et al. [16] suggested that the expression and activity of iron-management proteins, including transferrin and its receptor, in the choroid plexus and brain microvasculature in the brains of patients with RLS differ from that observed in healthy control subjects without RLS.

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Iron is an important modulator of DA neurotransmission [14]. However, the process by which disruption of brain iron homeostasis leads to alterations in DAergic neurotransmission and the development of RLS remains unknown. Iron deficiency can be reproduced experimentally in animal models, thus providing an opportunity to explore how changes in iron metabolism affect DAergic signaling pathways, putatively resulting in the development of RLS in humans. Nonetheless, there are other potential pathways by which an individual with otherwise normal-range iron levels and DA metabolism may develop RLS. Therefore, the iron–DA theory of RLS is a singular hypothesis for which additional causative factors or intervening systems, including adenosinergic [17], opioid [18], or glutamatergic [19] systems, may be involved pathologically in RLS. The purpose of this review is to examine and evaluate the evidence for one major conceptual framework for a biological basis of RLS: the iron deficiency–DA metabolic theory of RLS. It relates the findings from animal models investigating the influence of brain iron deficiency on DAergic systems to data from clinical studies in patients with RLS.

2. Animal models of brain iron deficiency and DA abnormalities in RLS

The iron-deficient (ID) rodent (mouse and rat) is a compelling animal model for RLS. It has permitted the evaluation of the pathway between iron deficiency and the DAergic system and has helped identify possible biological endpoints, including the role of iron-transport proteins and changes in DA receptor expression, which may underlie the disease pathology [20–29].

2.1. Iron deficiency and circadian iron dynamics

The diagnosis of RLS requires the presence of diurnal symptom variation. Whole-brain iron content in mice does not show significant diurnal change; there are, however, diurnal changes in iron metabolism that suggest brain-region- and sex-specific circadian changes [29]. Significant increases in the light (inactive) compared to dark (active) periods ($p < 0.05$) occur for transferrin receptor, the primary cellular iron importer in the ventral midbrain (VMB; containing the substantia nigra and ventral tegmental area) in both sexes [29]. Male but not female mice showed a marginally, nonsignificant increase in VMB iron during the light (inactive) phase compared with the dark (active) phase. However, female but not male mice showed a greater iron concentration in the nucleus accumbens ($p < 0.05$) during the light phase in relation to the dark phase [29]. These increases in iron concentration or transferrin receptor seen in the light phase were absent under a diet-induced, iron-deficiency condition. This suggests that brain iron deficiency may affect circadian-dependent iron homeostasis or cellular metabolism. As these diurnal measures only evaluate two time points, the nature of this iron-deficiency effect is unclear. The iron-deficiency effect may be a damping of the circadian oscillation or a shift in the phase of the oscillation but, without more time points, the current findings do not have a definite explanation.

The only study to evaluate iron changes over time and thus provide a true understanding of the circadian nature of iron utilized an indwelling microdialysis probe to measure in vivo extracellular iron in the substantia nigra over 3 days [30]. BXD, recombinant inbred, strain 40 female mice were maintained on an ID diet since weaning and were evaluated at about postnatal day 90. Extracellular, non-transferrin-bound iron demonstrated a distinct circadian pattern with the nadir in the early part of the dark cycle and the peak in the early part of the light cycle [30]. A week later, the mice were sacrificed and the brain iron levels show the expected low iron level in the substantia nigra. As all of the animals in the study were on an ID diet, a comparison with non-ID mice could not be made. Thus, it is not known whether iron deficiency had an effect on the observed circadian changes as was found in the diurnal studies. This study also differs from the diurnal study where whole tissue levels of iron or iron regulator protein were assessed while in this study in vivo extracellular iron concentration was determined. It is, however, clear from this one study that ID mice still have some component of the iron regulatory system within the substantia nigra that follows circadian biology.

2.2. Iron deficiency and DA dynamics

Iron deficiency in animals affects the brain DAergic system. Tyrosine hydroxylase (the rate-limiting enzyme in DA synthesis) and phosphorylated tyrosine hydroxylase (the activated form of tyrosine hydroxylase) concentrations in the caudate–putamen and in the VMB were found to be significantly higher in rats made iron deficient by dietary restriction than in control animals ($p < 0.001$) [23]. Iron chelation in cell cultures similarly produced increases in tyrosine hydroxylase and its phosphorylated form [23]. As iron is an essential cofactor for tyrosine hydroxylase activity, the increase in tyrosine hydroxylase was therefore opposite to the initial hypothesis (i.e., decrease in iron should result in decreased tyrosine hydroxylase) but similar to the postmortem finding in brains of patients with RLS (see Section 3.2 Autopsy studies). The basis of this increase may reside in the effects of iron deficiency on hypoxia pathway and the hypoxia pathway effects on tyrosine hydroxylase (see Section 4.1 Cellular factors).

Studies using microdialysis techniques to determine the concentration of DA and its metabolites in the extracellular space also support the concept of increased presynaptic DA activity with iron deficiency at least in the caudate–putamen. Using microdialysis techniques in ID rats, studies found a ≥50% increase in extracellular DA in the caudate–putamen ($p > 0.001$) and ≥30% increase in the DA metabolite, homovanillic acid ($p > 0.01$) [20,27]. The fact that cocaine, an inhibitor of the DA uptake transporter (DAT), in ID rats did not lead to the acute rise in extracellular DA seen in rats without ID suggests the increase in DA levels in ID animals may in part be a result of altered DAT function [20,27].

Using in vivo and in vitro biochemical methodologies as well as behavioral testing, Erikson et al. [24] provided the first evidence that the increase in extracellular caudate–putamen DA concentration in ID animals was due to decreased functioning of DAT. Another study found that ID rats had significantly lower mean cytosolic (35% lower; $p < 0.01$) and membrane-bound (73%; $p < 0.001$) DAT protein densities in the caudate–putamen compared with controls [22]. Such reductions in DAT protein density may indicate altered DAT trafficking in addition to the reduction in DAT production [22]. A later study demonstrated that iron deficiency has an effect on the diurnal changes in DAT density in mice. In normal-diet rats, the DAT binding in the caudate–putamen and in the nucleus accumbens was higher in the light, active phase. Iron deficiency led to a loss of that diurnal effect as was seen with diurnal changes in iron metabolism [21].

The most direct measures of intrasynaptic DA dynamic and DAT function in a live animal can be obtained using the no-net-flux (NNF) microdialysis technique. The NNF technique allows for the determination of extracellular neurotransmitters in live animals based on models of diffusion and catabolism [31,32]. One particular measure, the extraction fraction, provides real-time estimates of DAT-dependent removal of DA from the extracellular space and thus provides qualitative estimates of DAT function [33,34]. Bianco et al. (2008) [22] used the NNF to determine DA extrasynaptic dynamics in the striatum of rats. They determined that dietary iron deficiency led to an increase in extracellular DA, which resulted from a decrease extraction by DAT. The results of these studies suggest that iron deficiency strongly affects both presynaptic DA turnover