Mismanagement of iron homeostasis in iron-loaded *ex vivo* slice cultures

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Abstract

Recent studies have shown alterations in iron distribution and the expression of iron-related molecules in multiple sclerosis. A clearer picture of this metabolic dyshomeostasis of iron might be gained by establishing an ex vivo slice culture model of aberrant CNS iron homeostasis. Here, we examined the molecules involved by iron-loading rat organotypic slice cultures. We have demonstrated differential uptake and toxicity of iron after 12 h of exposure to 10 μM ferrous ammonium sulfate, ferric citrate or ferrocene. Moreover, 1 μM ferrocene produced the maximal 1.6-fold increase in iron content and increased expression of ferritin light-chain mRNA (an iron storage molecule). In summary, ferrocene loading perturbs iron metabolism in our hippocampal slice culture system. This model of iron loading appears to be a promising platform for studying iron regulation in the CNS.

1. Introduction

Aberrant iron metabolism and abnormal iron deposition in the brain are associated with a number of neurodegenerative disorders including multiple sclerosis (1). Recent studies carried out using human multiple sclerosis tissue, and an EAE animal model of spinal cord demyelination, have demonstrated increased iron accumulation that is localised in microglia and macrophages in active lesions and also a release of iron from oligodendrocytes upon demyelination (2-5). However, it remains unclear whether this metabolic dyshomeostasis of iron is causative or is a consequence of the disease process.

A clear picture of CNS iron handling might be gained using an ex vivo slice culture platform. Since existing iron loading studies are restricted to single cell populations, they cannot replicate the intricate iron handling relationships between cell types and lack the major advantage of ex vivo slices. Therefore, the successful iron loading in our novel and relevant ex vivo hippocampal slice culture model represents a major advancement in the clarification of how brain iron is regulated.

2. Methods

We generated slice cultures from P10-11 Sprague-Dawley rats and used ferrous ammonium sulfate, ferric citrate and ferrocene as iron-loading reagents at a concentration of 10 μM for 12 hr. Iron levels were quantified using a ferrozine colorimetric assay and viability was assessed using LDH and MTT assays. Using real-time PCR, the expression level of iron-storage molecule ferritin light chain was determined.

3. Results

Firstly, we demonstrated that iron content, as measured by a ferrozine colorimetric assay, in slice cultures after 10 days in culture (5.61±0.66 nmol/mg) replicates iron content in age-matched tissue samples (5.99±1.03 nmol/mg; $p>0.05$). Moreover, iron content was on a par with values reported for primary cultures of CNS cells. Secondly, we demonstrated differential iron uptake and toxicity after 12 hr exposure to 10 μM (a supraphysiological concentration) ferrous ammonium sulfate, ferric citrate or ferrocene. Thirdly, we showed that 1 μM ferrocene causes maximal iron loading and produced a 1.6-fold increase in iron content (4.97±0.57 to 8.05±0.98 nmol/mg; $p<0.05$; Fig. 1) with minimal impact on culture viability (as assessed by LDH and MTT assays). This iron accumulation was confirmed by detection of transcriptional upregulation of ferritin light-chain (an iron storage molecule).

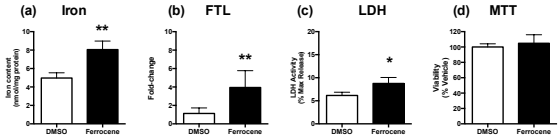

Figure 1: One μM ferrocene perturbs iron homeostasis and viability.

4. Conclusion

For the first time, we have demonstrated that iron-loading with ferrocene perturbs iron metabolism in *ex vivo* hippocampal slice culture system. We believe that this model of iron loading to be a promising platform for studying iron regulation in the CNS and will help us to understood the mechanism underlying iron accumulation in multiple sclerosis.

5. References

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