Available online at www.sciencedirect.com

SCIENCE DIRECT

Molecular Brain Research 133 (2005) 266–273

MOLECULAR BRAIN RESEARCH

www.elsevier.com/locate/molbrainres

Research report

Changed iron regulation in scrapie-infected neuroblastoma cells

Sandra Fernaeus[a], Jonas Hälldin[a], Katarina Bedecs[b], Tiit Land[a,*]

[a]Department of Neurochemistry and Neurotoxicology, Stockholm University, S-106 91 Stockholm, Sweden
[b]Department of Biochemistry and Biophysics, Stockholm University, S-106 91 Stockholm, Sweden

Accepted 17 October 2004
Available online 23 November 2004

Abstract

Prion diseases are characterized by the conversion of the normal cellular prion protein PrP^{C} into a pathogenic isoform, PrP^{Sc}. The mechanisms involved in neuronal cell death in prion diseases are largely unknown, but accumulating evidence has demonstrated oxidative impairment along with metal imbalances in scrapie-infected brains. In this study, we report changes in cellular iron metabolism in scrapie-infected mouse neuroblastoma N2a cells (ScN2a). We detected twofold lower total cellular iron and calcein-chelatable cytosolic labile iron pool (LIP) in ScN2a cells as compared to the N2a cells. We also measured in ScN2a cells significantly lower activities of iron regulatory proteins 1 and 2 (IRP1 and IRP2, respectively), regulators of cellular iron by sensing cytosolic free iron levels and controlling posttranscriptionally the expression of the major iron transport protein transferrin receptor 1 (TfR1) and the iron sequestration protein ferritin. IRP1 and IRP2 protein levels were decreased by 40% and 50%, respectively, in ScN2a cells. TfR1 protein levels were fourfold reduced and ferritin levels were threefold reduced in ScN2a cells. TfR1 and ferritin mRNA levels were significantly reduced in ScN2a cells. ScN2a cells responded normally to iron and iron chelator treatment with respect to the activities of IRP1 and IRP2, and biosynthesis of TfR1 and ferritin. However, the activities of IRP1 and IRP2, and protein levels of TfR1 and ferritin, were still significantly lower in iron-depleted ScN2a cells as compared to the N2a cells, suggesting lower need for iron in ScN2a cells. Our results demonstrate that scrapie infection leads to changes in cellular iron metabolism, affecting both total cellular and cytosolic free iron, and the activities and expression of major regulators of cellular iron homeostasis.

Theme: Disorders of the nervous system
Topic: Degenerative disease: other

Keywords: Prion; N2a cell; Iron regulation; Labile iron pool; Total cellular iron; IRP

1. Introduction

Prion diseases are infectious, inherited or sporadic neurodegenerative diseases, including Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease, kuru and fatal familial insomnia in humans. Animal prion diseases include scrapie in sheep and bovine spongiform encephalopathy. The main neuropathological features in prion diseases are spongiform degeneration, astrogliosis and neuronal cell death; however, the cause for and mechanism of neuronal loss is yet unknown [18]. The central feature of prion diseases is the posttranslational conversion of a normal host-encoded cellular prion protein (PrP^{C}) to an abnormal malfolded isoform (PrP^{Sc}). Normal prion protein is primarily expressed in neurons, glial cells and lymphocytes, but the precise function of PrP^{C} is largely unknown.

A growing body of evidence suggests that the PrP^{C} may be involved in maintaining the proper oxidative balance of the cell. It has been shown that primary neurons from PrP knockout mice ($PrP^{0/0}$) have the reduced copper–zinc superoxide dismutase (Cu/Zn-SOD) activity [1], and increased sensitivity to oxidative stress has been observed in scrapie-infected hypothalamic neuronal GT-1 cells [16], and in neuronal cells from both $PrP^{0/0}$ [26] and scrapie-

* Corresponding author. Tel.: +46 8 164169; fax: +46 8 161371.
E-mail address: tiit@neurochem.su.se (T. Land).

0169-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbrainres.2004.10.018

infected mice [28]. Additional indication that oxidative stress events are present in prion diseases is the presence of lipid peroxidation in brains of infected animals or in infected cell cultures [3,14,16,27]. Furthermore, it has been demonstrated that oxidative impairment in scrapie-infected mice is associated with significantly lower levels of divalent copper, zinc, magnesium and calcium in the brain [27], suggesting that brain metal imbalances, especially copper, in scrapie infection is likely to affect the antioxidant functions of PrPs. However, possible role of iron in oxidative impairment in scrapie-infected tissues or cells is not well studied, and conflicting results about changed iron levels in scrapie-infected brains have been reported. Kim et al. [13] have shown that the concentration of total iron (Fe) and ferric iron (Fe^{3+}) is increased in the brains of scrapie-infected mice, whereas Wong et al. [27] have measured significantly decreased ferrous iron (Fe^{2+}) concentrations in the brains of the 139A scrapie strain and unchanged Fe^{2+} levels in ME7 scrapie strain.

Iron is required for a wide variety of biological functions, ranging from oxygen transport and mitochondrial oxidation reactions to the synthesis of dopamine and DNA. However, free iron has the ability to catalyze through Fenton reaction the formation of highly reactive hydroxyl radicals that damage essential biological components, such as DNA, proteins and lipids. It is well known that a misregulation of cellular iron homeostasis, iron-induced oxidative stress and free radical formation are major pathogenic factors, and iron accumulation in specific regions of the brain is observed in several neurodegenerative diseases, including Hallervorden–Spatz syndrome [21], Parkinson's disease [5,10], Alzheimer's disease [20] and Friedreich's ataxia [24]. It is likely that misregulation of iron metabolism is important in the pathophysiology of several neurodegenerative diseases.

Intracellular iron homeostasis is maintained by regulatory proteins that sense intracellular free iron levels. In mammalian cells, there are two cytosolic iron regulatory proteins, iron regulatory protein 1 and iron regulatory protein 2 (IRP1 and IRP2, respectively) that in iron-depleted cells bind to RNA stem-loop structures known as iron-responsive elements (IREs) found within transcripts of iron transport protein transferrin receptor 1 (TfR1) and iron sequestration protein ferritin, decreasing the turnover rate of TfR1 transcript and the translation rate of ferritin transcript, resulting in higher TfR1 and lower ferritin protein levels (for a review, cf. Refs. [9,19]). When cells are iron replete, IRP1 acquires an iron–sulfur cluster prosthetic group resulting in the loss of its IRE-binding activity [8,12], whereas IRP2 undergoes ubiquitination and proteasomal degradation [6,11].

In this report, we have measured iron levels and studied the activities and expression of key proteins involved in intracellular iron regulation in scrapie-infected mouse neuroblastoma N2a cells (ScN2a). We demonstrate that ScN2a cells contain twofold lower total cellular iron and labile iron pool (LIP) as compared to the N2a cells. We also show that the activities and protein levels of IRP1 and IRP2, and protein and mRNA levels of TfR1 and ferritin are significantly reduced in ScN2a cells.

2. Materials and methods

2.1. Materials

Anti-IRP1, anti-IRP2 and antiferritin antibodies were generously provided by Dr. Tracey A. Rouault. Anti-TfR antibodies were purchased from Zymed Laboratories. Secondary horseradish peroxidase conjugated goat antirabbit IgG and antimouse IgG were purchased from Amersham Pharmacia Biotech. TRIZOL® Reagent and all cell culture reagents were from Invitrogen. Calcein-AM was purchased from Molecular Probes. All other reagents were from Sigma.

2.2. Cell culture and cell treatment

ScN2a cells were generated as previously described [2] and together with noninfected N2a cells were generously provided by Dr. Stanley B. Prusiner. N2a cells were also purchased from ATCC (Rockville, MD). The cells were maintained at 37 °C under an atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) with Glutamax II and 4.5 g/l D-glucose supplemented with 5% fetal bovine serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. The ScN2a cells were routinely checked for their scrapie infection as described by Östlund et al. [17]. For iron and iron chelator treatments, cells were treated for 16 h with either ferric ammonium citrate (FAC; 100 μg/ml) or desferrioxamine (DFO; 100 μg/ml).

2.3. Cellular iron measurements

Total cellular iron was measured as described [22]. Briefly, samples of 5×10^6 cells were suspended in 1 ml HBS buffer (150 mM NaCl, 20 mM HEPES), mixed with 1 ml of an acid mixture [3 M HCl, 10% (v/v) trichloroacetic acid, 3% (v/v) thioglycolic acid], incubated at 37 °C for 2 h, cooled, centrifuged at $3000\times g$ for 30 min and mixed with 0.2 ml bathophenantroline sulfonate [0.045% (w/v) in 4.5 M Na-acetate, 0.2% (v/v) thioglycolic acid]. The colored iron–bathophenantroline sulfonate complex was measured by determining the absorption at 535 nm.

2.4. LIP measurements

The cytosolic labile iron pool (LIP) was determined as described [4]. Briefly, 4×10^6 cells were loaded with 100 nM calcein-AM in PBS at 37 °C for 15 min. After washing noninternalized calcein, the cells were transferred to a stirred, thermostated cuvette, and the basal calcein fluorescence was recorded (excitation 488 nm, emission 517