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The effects of prion protein expression on metal metabolism

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ABSTRACT

The prion protein is a glycoprotein that binds metals such as copper and manganese. When converted to a proteinase resistant isoform it is associated with prion diseases such as Creutzfeldt–Jakob disease and bovine spongiform encephalopathy. Although, the co-ordination and metal affinity of the prion protein has been well studied, the association of the protein with cellular metal metabolism has been less well investigated. We used transgenic manipulation of prion protein expression and other recombinant techniques to alter expression of known copper binding proteins to investigate the role of the prion protein in copper metabolism. We found that changing the expression of the prion protein alters proteins associated with copper uptake, storage and export from the cell. In addition, alteration in the expression of superoxide dismutases increased prion protein expression dramatically. Reducing copper in the diet decreased expression of the prion protein in the brain while increased dietary manganese dramatically increased the protein's expression. Cellular prion infection also increased the expression of metal transporting proteins and increased cellular manganese concentrations. Overall our results show a close link between cellular resistance to oxidative stress and also copper metabolism. These findings are in line with previous data suggesting that the prion protein is an antioxidant and associated with copper uptake into cells. The disturbance to copper metabolism, as a result of altered prion protein expression clearly demonstrates the important role of the prion protein in copper metabolism. The implication is that prion protein expression has a homeostatic role in copper metabolism.

Introduction

Prion disease or transmissible spongiform encephalopathies are a family of neurodegenerative disorders (Prusiner, 1998). These include Creutzfeldt–Jakob disease, scrapie and bovine spongiform encephalopathy. The diseases include inherited, sporadic and transmitted forms but are all associated with the misfolding of a single protein termed the prion protein (Bolton et al., 1982). While diseases are associated with the deposition of an abnormal isoform of the prion protein (PrP^{Sc}), the normal protein (PrP^{c}) occurs at high concentrations in the brain, particularly at synapses. Deciphering the conversion process from the normal cellular form to PrP^{Sc} is key to understanding, preventing and treating prion diseases (Cohen and Prusiner, 1998), as is a thorough understanding of the normal role of PrP^{c} in the cell (Brown, 2001).

PrP^{c} is a glycoprotein anchored to the external surface of the cell membrane by a glycosylphotidyl inositol (GPI) anchor (Stahl et al., 1992). The protein is glycosylated at two asparagine residues and contains a disulfide bridge (Multhaup et al., 1985; Turk et al., 1988). The protein when free of metals is highly structured at the C-terminus whereas in solution the N-terminus is unstructured. An octameric repeat region is present within the N-terminus and considered to be the main binding site for copper and possibly other metals such as manganese and zinc (Brown et al., 1997a, 2000). Additional binding sites for copper are located near the octameric repeat region (His 95 and 110) and termed the "5th Site" as four copper ions can bind to the octameric repeat region (Jones et al., 2004). Although there is good evidence that the normal metal binding partner for PrP is copper, there is also strong evidence that manganese can bind to PrP and can cause its conversion to an altered isoform (Brown et al., 2000; Brazier et al., 2008). Studies have suggested a variety of possible structural consequences upon copper binding. Some suggest minor changes while other data suggest significant unfolding of the protein. Additionally, there has been considerable variability in observed affinity values for copper binding, ranging from micromolar to femtomolar, with the former affinity thought to probably be the most biologically plausible (Hornshaw et al., 1995, Brown et al., 1997a; Kramer et al., 2001: Jackson et al., 2001; Thompsett et al., 2005). Similarly, there has been intense debate over the possible function of

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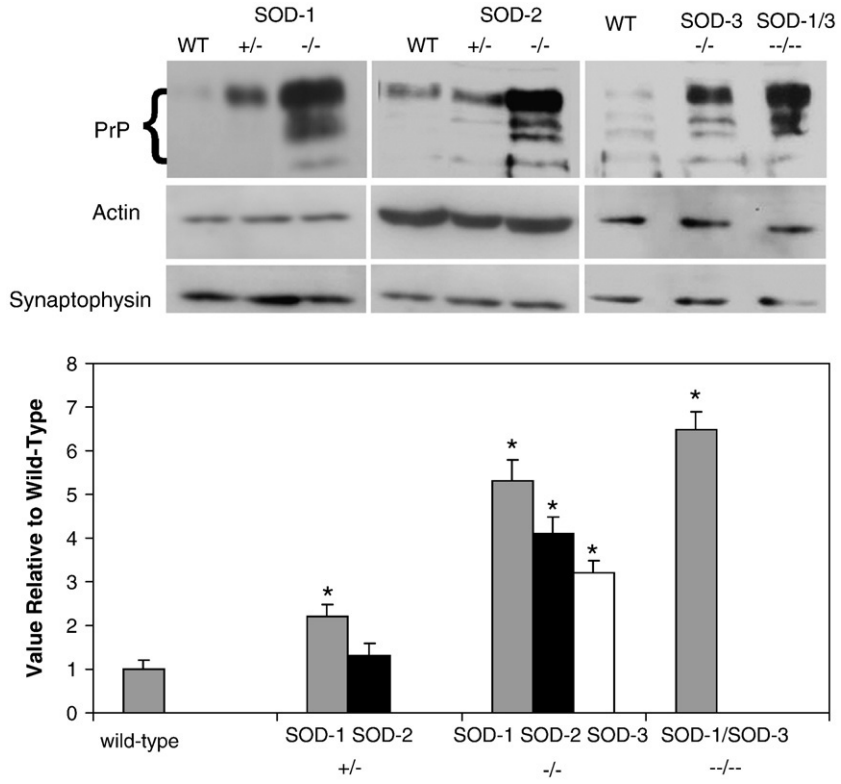

Fig. 1. PrP expression in SOD knockout mice. The brains of heteroxygotic and homozygotic knockout mice for SOD1 (Cu/Zn SOD), SOD2 (MnSOD) and SOD3 (EC-SOD) as well as the double homozygous knockout for SOD1 and SOD3 were compared to those of wild-type mice in terms of PrP expression levels. Brains were homogenised and equal amounts of protein extracts electrophorese on a PAGE gel. Following western blotting and immunodetection, bands for PrP were detected and quantitated. Two other proteins analysed in parallel were synaptophysin and actin. While no changes were observed for the two control proteins significant changes were observed for PrP. Examples of the blots are shown in the upper panels while the lower panel shows the mean and standard error for densitometric analyses of blots from five different brains for each mouse type in the lower panel. Values are compared to those of the wild-type mice as fold increase or decrease. * Indicates measurements significantly different to wild-type ($p<0.05$). All homozygote knockout values were significantly greater than those for heterozygotes ($p<0.05$) and the double knockout had significantly higher levels of PrP than either of the single knockouts ($p<0.05$).

the protein, with some suggestions unrelated to the metal binding capacity. In contrast, there is quite a lot of evidence that the metal binding is associated with a cellular function that protects cells from stress. The protein could act as a copper sensor or as an antioxidant. Specifically, it has been suggested that PrP^c could act as a superoxide dismutase (SOD) (Brown et al., 1999, 2001). Although there are some findings contradicting a SOD-like role for PrP^c (Sakudo et al., 2008), the majority of evidence suggests the protein serves to protect cells.

PrP^c can be isolated from brains with copper still bound (Brown et al., 2001). Knockout of expression of PrP^c results in decreased levels of copper at synaptosomes (Brown, 2003) and decreases copper uptake into cells (Brown, 1999, 2004) while exposure of cell lines to increase copper concentrations results in cells with constitutively high levels of PrP^c expression (Brown et al., 1997b). Knockout of PrP^c expression has other effects including reducing cellular resistance to oxidative stress and causing reduction in the levels of active Cu/Zn superoxide dismutase (Brown and Besinger, 1998). The importance of copper in maintaining the normal structure of PrP^c has come from studies showing that added copper inhibits aggregation of the protein (Bocharova et al., 2005) and delays disease progression (Hijazi et al., 2003). In contrast to this, other studies have shown that copper chelation can also delay disease progression (Sigurdsson et al., 2003). It is possible that once PrP begins to aggregate as the abnormal isoform, copper has the opposite effect to that seen when normal copper binding to the octameric repeat region is maintained. Understanding the role of copper binding to PrP and the consequences of disease progression may require a better understanding of the role of PrP in copper and perhaps metal metabolism overall.

In this study we have looked at the relationship between PrP^c and a large number of other proteins involved in metal metabolism and antioxidant protection. We show that the level of expression of PrP^c is dependent on availability of copper and is altered by the expression of other superoxide dismutases. Furthermore altering the expression of PrP^c causes changes to a number of other copper binding proteins, indicating an important role for the protein is copper metabolism. Our data provides a greater definition to the cellular role of PrP^c as a stress detector protein important for cellular copper homeostasis.

Results

In this study we examined the relationship between proteins that bind copper or manganese and prion protein expression. Initially, we examined the expression of either PrP or metalloproteins in the brains of transgenic mice with altered protein expression.

PrP and SOD knockout mice

Superoxide dismutases are both antioxidants and metal binding proteins. The relative level of expression of PrP in the brains of mice lacking expression of one of three well characterised SODs was studied using western blot. The three SODs studied were Cu/Zn SOD (SOD1), MnSOD (SOD2) and extracellular-SOD (SOD3). Both homozygous and heterozygous SOD1 ($SOD1^{-/-}$ and $SOD1^{+/-}$, respectively) and SOD2 ($SOD2^{-/-}$ and $SOD2^{+/-}$, respectively) knockout mice were analysed, as well as homozygous SOD3 knockout mice ($SOD3^{-/-}$) and a double knockout for SOD1 and SOD3 ($SOD1/SOD3^{--/--}$). For comparison, levels of actin and synaptophysin were also analysed. The absence of expression of the SOD proteins was confirmed by western blotting (data not shown).