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Research Report

The neurochemical nature of PrP^C-containing cells in the rat brain

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ABSTRACT

The cellular prion protein (PrP^C) is a membrane-bound glycoprotein abundantly expressed in neurons and glial cells within the CNS. The scrapie prion protein (PrP^{Sc}) is a conformationally altered isoform of PrP^C that is responsible for prion diseases, also termed transmissible spongiform encephalopathies (TSE), a group of neurodegenerative diseases that affect a wide variety of mammal species, including humans. The presence of the cellular isoform of PrP is necessary for the establishment and further evolution of prion diseases and the physiological conditions where PrP^C is present seems to modulate the alterations in TSE. In this work, the presence of PrP^C in GABAergic, glutamatergic, nitrergic, cholinergic, serotoninergic and orexinergic populations of cells within the rat brain is examined. Our observations show that PrP^C is widely expressed in a subset of neurons that contain markers of inhibitory populations of cells throughout the rat brain. The presence of PrP^C in other cells types containing important neurotransmitters for the overall brain function is congruent with the imbalances reported for some of them in TSE. Within the cerebral cortex, PrP^C is scarcely located in a subset of cells expressing the laminin receptor precursor (LRP) to such a low extent that suggests that other LRP-independent mechanisms actively participate during the pathogenic process. Taken together, our data demonstrate that investigation of the chemical partners of PrP^C within cells gives a rational basis for the interpretation of the histopathological alterations in TSE and might help analyze some pathogenic mechanisms of PrP^{Sc}.

1. Introduction

Transmissible spongiform encephalopathies (TSE) represent a heterogeneous group of neurodegenerative conditions that affect both animals and humans (Prusiner, 1998). They are produced by the appearance of the *scrapie* isoform of the prion protein (PrP^{Sc}, also termed prions), a pathogenic and posttranslationally altered isoform of the cellular form of the prion protein (PrP^C), a membrane-bound glycoprotein attached to the cell surface by a GPI anchor (Stahl et al., 1987) that is ubiquitously expressed throughout many tissues and cell types in all the animal species where TSE occur, but especially abundant in neurons (Bendheim et al., 1992; Kretzschmar et al., 1986). In spite of the multiorganic presence of PrP^C within mammals, its function remains to be determined as the study of knockout mice for PrP ($PrP^{-/-}$) has not shed sufficient light on this issue (Aguzzi and Polymenidou, 2004). Other experimental approaches have shown that PrP^C might be involved in neurogenesis and

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Abbreviations: CB, calbindin; CBP, calcium-binding proteins; CJD, Creutzfeldt-Jakob disease; CR, calretinin; FFI, Fatal Familial Insomnia; PNN, perineuronal nets; PrP^C, cellular prion protein; PrP^{Sc}, *scrapie* prion protein; PV, parvalbumin; TSEs, transmissible spongiform encephalophaties

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differentiation, neuroprotection and cell signaling (Steele et al., 2006; Vassallo and Herms, 2003), although the complete knowledge to fully understand the unified function of this protein needs further examination. PrP^C has been shown to be necessary for the establishment and further evolution of TSE (Bueler et al., 1993). According to the "protein-only hypothesis", postulated by Standley Prusiner in 1982 (Prusiner, 1982), PrP^{Sc} interacts with PrP^C and acts as a template for the conversion of PrP^C into a *de novo* generated PrP^{Sc} within the cell that had exogenously acquired that first molecule of PrP^{Sc}. The appearance of this molecule of PrP^{Sc} in the organism is used to classify TSE as: (i) acquired TSE (mainly due to dietary intake of PrP^{Sc} or contamination through different exposures to the agent), such as kuru, new variant Creutzfeldt–Jakob disease (vCJD), or iatrogenic CJD (iCJD); (ii) familial TSE (mutations in the coding region of PRNP, the gene that encodes PrP^C, that under these circumstances generates mutant, abnormally folded, PrP), such as Gerstmann–Sträussler–Scheinker (GSS), familial CJD (fCJD), or fatal familial insomnia (FFI); (iii) sporadic TSE (unknown origin, a rare stochastic event that produces the abnormal folding of PrP), such as sporadic CJD (sCJD, the most common form of human TSE) and sporadic fatal insomnia (sFI) (Prusiner, 1998). Each TSE is produced by a different strain of PrP^{Sc}, which determines its phenotype (Parchi et al., 2000).

TSE can be confirmed by the different physicochemical properties of the two isoforms of PrP (Meyer et al., 1986; Oesch et al., 1985) and by several neuropathological features as spongiosis and PrP^{Sc} deposition. Other non-TSE histological features like gliosis and neuronal loss are generally present (Budka, 2003). Although the presence and extension of these histological features are variable, the neuronal loss seems to be specific to some particular subsets of cells within the affected brains. Hence, a decrease in the number of a population of inhibitory neurons has been consistently described in human and experimental TSE (Belichenko et al., 1999; Bouzamondo-Bernstein et al., 2004; Bouzamondo et al., 2000; Durand-Gorde et al., 1984, 1985; Ferrer et al., 1993; Gregoire et al., 1993; Guentchev et al., 1998, 1997, 1999; Lu et al., 1995; Tschampa et al., 2002). Since the expression of PrP^C is necessary but not sufficient for PrP^{Sc} replication, the question of what makes PrP^{Sc} damage some subsets of cells and leave others unchanged remains to be experimentally answered. Hence, as the neuronal vulnerability to PrP^{Sc} seems to be influenced by the chemical nature of a particular neuron, its investigation will set a basis for the explanation of the pathogenic mechanisms in TSE.

Parvalbumin (PV), calbindin (CB) and calretinin (CR) are three types of calcium-binding proteins (CBP) that are physiologically expressed in non-overlapping populations of GABAergic cells within the cerebral cortex (Celio, 1986). PV-positive neurons are particularly affected in most forms of TSE (Ferrer et al., 1993), mainly those surrounded by perineuronal nets (PNN) (Belichenko et al., 1999; Guentchev et al., 1998), a specialized form of extracellular matrix which surrounds a subset of PV-containing cells (Celio and Blumcke, 1994). In addition to the prominent loss of inhibitory cells within the brain of TSE affected subjects, the alteration of other systems has also been demonstrated, as shown by the alteration of serotoninergic (Ledoux, 2005), glutamatergic (Rodríguez et al., 2005, 2006) and cholinergic systems (McDermott et al., 1978; Rubenstein et al., 1991). Currently there is no explanation for this selective neuronal loss, although several hypotheses have emerged in an effort to determine the molecular basis of this cellular decrease. However, some of these models of PrP^{Sc} pathogenesis are based on neuropathological recordings and do not take into account the neuronal substrate where PrP^C is expressed, a limiting factor stated by the "protein-only hypothesis". Therefore, the investigation of the chemical nature of the neurons that contain PrP^C is a key step for the correct interpretation and understanding of the histolopathological aspects of TSE.

In a previous work, we quantified the levels of expression of PrP^C, analyzed its localization and described the distribution pattern of PrP^C in CBP-containing inhibitory cells of the cerebral cortex (Moleres and Velayos, 2005). This study helped us propose a pathogenic mechanism of PrP^{Sc} that can explain the inhibitory cellular loss in TSE, hence demonstrating that the characterization of PrP^C-positive cells is a key step to interpret, within a physiological background, the histological findings of TSE (Moleres and Velayos, 2005). In the present work, we have increased the number of neuronal markers for the characterization of cortical PrP^C and have also analyzed the biochemical nature of the cells where PrP^C is located.

2. Results

2.1. *Biochemical characterization of the PrP^C-containing neurons in the cerebral cortex*

In our previous work, we demonstrated that PrP and CBP displayed a prominent collocation within the cerebral cortex. Additionally, we found that PrP was present in a subset of PNN-surrounded cells (Moleres and Velayos, 2005). In this work, further characterization of these PrP- and PNN-immunopositive cells reveals that both markers are co-expressed in cells that also contained LRP, a protein present in a subset of cells surrounded by PNN (Fig. 1).

The use of additional markers such as vGLUT demonstrates that, in addition to this widespread presence in inhibitory cortical cells, there is a subset of PrP-positive cells surrounded by the presynaptic terminals labeled by this marker of excitatory neurons (Fig. 2A). This collocation is more abundant in the frontal cortex, although it is also present in other cortical territories, likely representing projection neurons and being clearly differentiated from the inhibitory (PNN-surrounded) interneurons previously shown in this and in our previous work (Moleres and Velayos, 2005).

In addition to GABAergic and glutamatergic markers, we have also analyzed the presence of PrP in glial cells and in nitrergic neurons. Thus, we have noted the presence of some astrocytes and oligodendrocites positively labeled for PrP throughout the different layers of the cerebral cortex where PrP is expressed (Fig. 2B). Within nitrergic neurons, PrP is present in some nNOS-immunoreactive cells throughout the different layers of the cerebral cortex, although this co-expression is slightly higher in layers II–III of the visual cortex (Fig. 2C), layers where the expression of PrP is more prominent within this cortical region.

2.2. *Cortically derived regions and subcortical areas*

The study of the hippocampus displays that PrP is abundantly expressed within inhibitory cells containing either PV (some of