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EXPERIMENTALLY INDUCED DISEASE

Mapping of Neurotrophins and their Receptors in the Adult Mouse Brain and their Role in the Pathogenesis of a Transgenic Murine Model of Bovine Spongiform Encephalopathy

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Summary

Neurotrophins are a family of growth factors that act on neuronal cells. The neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3, -4 and -5. The action of neurotrophins depends on two transmembrane-receptor signalling systems: (1) the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors (Trk A, Trk B and Trk C) and (2) the p75 neurotrophin receptor ($p75^{NTR}$). The interaction between neurotrophic factors and their receptors may be involved in the mechanisms that regulate the differential susceptibility of neuronal populations in neurodegenerative diseases. The aim of the present study was to evaluate the role of neurotrophins in the pathogenesis of bovine spongiform encephalopathy (BSE) using a transgenic mouse overexpressing bovine *prnp* (BoTg 110). Histochemistry for *Lycopersicum esculentum* agglutinin, haematoxylin and eosin staining and immunohistochemistry for the abnormal isoform of the prion protein (PrP^{d}), glial fibrillary acidic protein (GFAP), NGF, BDNF, NT-3 and the receptors Trk A, Trk B, Trk C and $p75^{NTR}$ was performed. The lesions and the immunolabelling patterns were assessed semiquantitatively in different areas of the brain. No significant differences in the immunolabelling of neurotrophins and their receptors were observed between BSE-inoculated and control animals, except for $p75^{NTR}$, which showed increased expression correlating with the distribution of lesions, PrP^{d} deposition and gliosis in the BSE-inoculated mice.

Keywords: brain; BSE prion; immunohistochemistry; neurotrophins

Introduction

Transmissible spongiform encephalopathies (TSEs or prion diseases) are a group of fatal neurodegenerative diseases affecting both man and animals and are characterized by having a long incubation period (Beringue *et al.*, 2008). This group of diseases can be sporadic, genetic or acquired, but they are all transmissible and have a common feature, which is the accumulation in the brain of an abnormal form of the host-encoded cellular prion protein (PrP^{C}). Additional to the deposition of disease-associated prion protein (PrP^{d}) in the brain, the main neuropathological features are spongiform change in the neuropil, vacuolation of neuronal bodies and astrocyte and microglial cell activation and neuronal loss (Della-Bianca *et al.*, 2001).

Among the animal TSEs, one of the best known is bovine spongiform encephalopathy (BSE), which was first reported in cattle in the mid 1980s (Wells and Wilesmith, 1995) and has had major

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public health implications as it is a food-borne zoonosis resulting in the invariably fatal variant Creutzfeldt–Jacob disease (vCJD) (Wilesmith *et al.*, 1988; Bruce *et al.*, 1997).

Neurotrophins are a family of structurally and functionally related proteins consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3, -4 and -5. They are synthesized as precursors (proneurotrophins) by both neuronal and non-neuronal cells prior to being either cleaved intracellularly by proconvertases or secreted in the unprocessed form. In the latter case, there is conversion through proteolytic cleavage by plasmin or other extracellular proteases to the mature form (Bartkowska *et al.*, 2010). In the nervous system of vertebrates, neurotrophins control many aspects of embryonic development (e.g. cellular survival, differentiation, plasticity and regeneration) and the functions of most adult neurons (Skaper, 2008).

The action of neurotrophins depends on two transmembrane-receptor signalling systems: (1) the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors (Trk A, Trk B and Trk C) and (2) the p75 neurotrophin receptor ($p75^{NTR}$), a member of the tumour necrosis factor receptor superfamily (Chao, 2003; Bartkowska *et al.*, 2010). Different neurotrophins show binding specificity for particular receptors. NGF binds preferentially to Trk A, BDNF and NT-4 to Trk B and NT-3 to Trk C. These interactions are considered to be of high affinity, but can be regulated by receptor dimerization, structural modifications or association with $p75^{NTR}$. The $p75^{NTR}$ can bind to all neurotrophins and also acts as a co-receptor with Trk receptors. Proneurotrophins are also active ligands of Trk receptors, but their binding elicits functional effects opposite to those elicited by the binding of mature neurotrophins (Chao, 2003; Reichardt, 2006).

The interaction between neurotrophic factors and their receptors is involved in the mechanisms that regulate the differential susceptibility of neuronal populations in neurodegenerative diseases (Connor and Dragunow, 1998). In prion diseases, PrP 106–126, a synthetic peptide homologous to the human PrP region 106–126, induces apoptosis in mouse neuroblastoma N2a cells, involving $p75^{NTR}$ and the nuclear factor-κB (NF-κB) signalling pathway (Della-Bianca *et al.*, 2001; Bai *et al.*, 2008). This suggests that neurotrophin receptors, and particularly $p75^{NTR}$, might be involved in prion disease pathogenesis. However, it has not been possible to find further publications on the subject.

The aim of the present study was to evaluate the role of neurotrophins and their receptors in a transgenic murine model (BoTg 110) of BSE. This transgenic mouse line is characterized by the overexpression (up to eight times the expression of a normal cow brain) of bovine *prnp* on a murine PRNP-knockout background (Castilla *et al.*, 2003) and has been shown to be a good model for study of the pathogenesis of BSE (Costa *et al.*, 2007, 2009; Espinosa *et al.*, 2007; Tortosa *et al.*, 2008, 2011). Little information was found regarding immunohistochemical investigations of NTs and NTRs in the mouse brain (Yan *et al.*, 1997a; Zermeno *et al.*, 2009; Bartkowska *et al.*, 2010; Parkhurst *et al.*, 2010), thus the study was performed in parallel with a wild type mouse line (Balb-C) to ensure that the transgene did not have an influence on the studied molecules and to establish a baseline immunolabelling pattern in paraffin wax-embedded samples of mouse brain.

Materials and Methods

Animals and Inoculum

A case of BSE was identified within the BSE active surveillance plan and characterization of this case has been described elsewhere (BSE case 1; Vidal *et al.*, 2005, 2006). An inoculum was prepared from this case. The Log_{10} lethal dose 50 (LD_{50}) for the inoculum per 20 µl was 4.9 (i.e. brain homogenized and diluted at $10^{-4.9}$) as determined by bioassay in BoTg 110 mice. All procedures were approved by the Animal Experimentation Ethics Committee of the Autonomous University of Barcelona (procedure number 585-3487).

A total of 14 female BoTg 110 mice were used for the neurotrophin study and were divided into two groups: those inoculated with BSE inoculum ($n = 8$; at a 1 in 10 dilution) and the control group ($n = 6$) inoculated with a healthy cow brain homogenate at 1 in 1,000 dilution. Each 6–8-week-old mouse received a 20 µl intracerebral inoculation.

Animals from an additional mouse model were used to perform the neurotrophin study. These were Balb-C wild type (WT) mice and 10 healthy non-inoculated females, 367 days old, were included.

Sample Processing

When scored positive for clinical BSE, mice were killed in accordance with the recommendations of the ethics committee. At necropsy examination, brain tissue was collected and placed in 10% neutral buffered formalin. Transverse sections were taken at three different levels of the brain (optic chiasm, piriform cortex and medulla oblongata) and these were processed routinely prior to being embedded in paraffin