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Changes in sympathetic activity in prion neuroinvasion

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ABSTRACT

Prion diseases are neurodegenerative diseases affecting humans and animals in which the infectious agent or prion is PrP^{res}, a protease-resistant conformer of the cell protein PrP. The natural transmission route of prion diseases is peripheral infection, with the lymphoreticular system (LRS) and peripheral nerves being involved in animal models of scrapie neuroinvasion and human prion diseases. To study the effects of PrP neuroinvasion on sympathetic nerve function, we measured plasma catecholamine levels, blood pressure, heart rate, and PrP tissue levels in intraperitoneally or intracerebrally infected mice. The results indicate a specific alteration in sympathetic nerve function because the levels of noradrenaline (but not adrenaline) were increased in the animals infected peripherally (but not in those infected intracerebrally) and correlated with increased blood pressure.

These findings confirm that prion neuroinvasion uses the sympathetic nervous system to spread from the periphery to the central nervous system after invading the LRS.

Introduction

Prion diseases are fatal animal and human neurodegenerative diseases in which the infectious agent or "prion" (Prusiner, 1982) is thought to be the same as PrP^{res}, a protease-resistant conformer of the cell protein PrP (Prusiner, 1982; Aguzzi and Weissmann, 1997).

Although prions are most efficiently propagated by means of intracerebral (i.c.) inoculation, the natural route of transmission of most prion diseases is peripheral infection. Kuru, bovine spongiform encephalopathy (BSE), and variant Creutzfeldt–Jakob disease (vCJD) are all caused by oral prion intake, whereas the parenteral administration of growth hormone and gonadotropins has led to iatrogenic Creutzfeldt–Jakob disease (Glatzel and Aguzzi, 2000a).

The pathways by which prions invade the central nervous system (CNS) are only partially understood but a large body of evidence indicates that the lymphoreticular system (LRS) and peripheral nerves are both involved in animal models of scrapie neuroinvasion (Klein et al., 1997; Lasmezas et al., 1996; Race et al., 2000), as well as in human prion diseases such as vCJD (Haïk et al., 2003). The mode of transport of peripheral nerve infection has not yet been determined, although recent studies suggest that prions use mechanisms other than conventional axonal transport (Glatzel and Aguzzi, 2000b; Hainfellner and Budka, 1999) that may involve PrP^{res}-specific binding proteins (Fischer et al., 2000). After intraperitoneal (i.p.) prion inoculation, pathological lesions and prion replication first appear in the segments of the thoracic spinal cord corresponding to the entry sites of the splanchnic nerves belonging to the sympathetic nervous system (SNS) (Cole and Kimberlin, 1985; Beekes et al., 1996), and prions also accumulate in the sympathetic ganglia (Felten and Felten, 1988).

As lymphoid organs are predominantly innervated by sympathetic nerve fibres, the SNS seems to be a prime target for prion transport and, possibly, replication (Bencsik et al., 2001; McBride and Beekes, 1999). It is known that lymphoid organs (including the spleen) are early sites of prion accumulation and replication after i.p. inoculation in animals (Eklund et al., 1967; Kimberlin and Walker, 1989) and in Creutzfeldt–Jakob disease (Glatzel et al., 2003). Prion neuroinvasion is suppressed by B-cell ablation (Klein et al., 1997) probably because B cells transmit a lymphotoxin-dependent signal to follicular dendritic cells (FDCs) (Montrasio et al., 2000), which are major sites of PrP^{res} deposition (Kitamoto et al., 1991). Finally, Glatzel et al. (2001) have demonstrated that peripheral sympathetic nerves play a major role in neuroinvasion by showing that the spread of PrP is markedly reduced in permanently sympathectomised mice.

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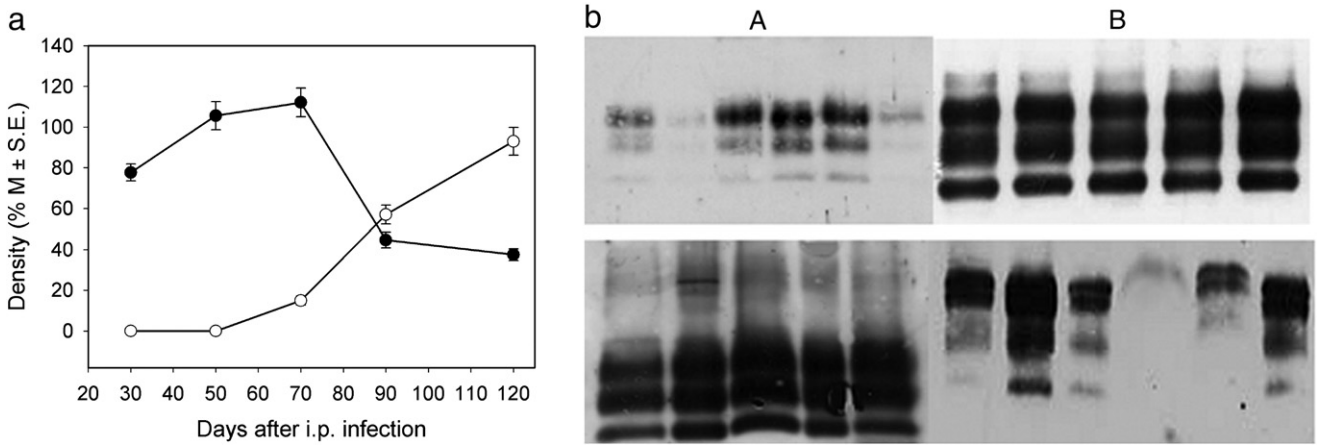

Fig. 1. (a) Western blots: time course of PrP in i.p. infected mice (values are expressed as the percentage of the density of the specific PrP^{res} signal produced by the reference sample): spleens, closed circles; brains, open circles. (b) Western blots of PrP^{sc}: 70 (A) and 120 days (B) after i.p. infection. Upper panels, brain; lower panels, spleen.

Preliminary experiments in hamsters have shown a marked increase in plasma noradrenaline 90 days after intraperitoneal (but not intracerebral) infection (Bareggi et al., 2006; Pollera et al., 2007).

In order to study the effects of PrP neuroinvasion on sympathetic nerve function, we determined plasma catecholamine levels in mice infected i.p. or i.c. at different times; at the same times, we also determined blood pressure, heart rate, and tissue PrP concentration.

Methods

Animals

All of the experimental procedures complied with the EU International Guidelines for animal experimentation, as well as with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

The male CD-1 mice aged 3–4 weeks and weighing 14–16 g (Charles River, Calco, Como, Italy) were housed in a conditioned environment (22 ± 1 °C, 55 ± 5% relative humidity, 12-h light/dark cycles) and fed *ad libitum* standard laboratory chow and water.

Experimental design

The animals were randomly divided into groups depending on the i.p. or i.c. route of infection by the RML strain of the mouse scrapie agent (Rocky Mountain Laboratories). A 10% (wt./vol.) homogenate of RML-infected CD-1 brain in phosphate-buffered saline was diluted to a final concentration of 1%, and 50 μl or 25 μl of the suspension was injected i.p. or i.c.. The control animals in each experiment were inoculated i.c. or i.p. with equivalent dilutions of non-infected mouse brain.

The animals were bled from the eye using a Pasteur pipette containing 10 μl of heparin within 60″ of isoflurane anesthesia taking effect. After centrifugation (3500 × *g* for 15 min at 4 °C), the plasma was separated and stored at −80 °C until being analysed for catecholamines (CAs).

Immunochemistry and histopathology

The animals were killed, and their brains and spleens were removed and PrP was extracted using the method described by Wadsworth et al. (2001) and, after proteinase K (PK, 50 μg/ml) treatment, were analysed for PrP by means of Western blotting (WB), with SAF 70 monoclonal antibody (Spi-bio, Montigny le Bretonneux, France) diluted 1:500 being used to detect PrP^{res}. The values were calculated as areas and density of the blots by means of densitometry analysis (Quantity One®, Bio-Rad Lab., Hercules, CA, USA) of band intensity in order to make a quantitative evaluation of PrP^{res} content in comparison with terminal brain-derived PrP^{res}. The results are expressed as the percentage of the density of the specific PrP^{res} signal produced by the reference sample.

The brains of the infected animals were also investigated for the presence of histopathological alterations after hematoxylin and eosin staining. The lesion profile in each area was examined by three independent observers, who gave a score from 0 to 4 depending on the density of the observed vacuolisation and spongiosis (none to severe).

Groups of eight i.c. infected mice were killed 30, 60, 90, and 120 days post-infection. They were anesthetised and decapitated, and their brains were rapidly removed, cut longitudinally into two parts, and immediately frozen on dry ice. Spleens were also taken and stored at −80 °C until analysis. Blood was drawn from the eye 30, 60, 90, and 120 days post-infection. In the case of the i.p. infected animals, groups of eight mice were killed 30, 60, 90, 120, and 180 days post-infection, when the same tissues were sampled and blood was drawn from the eye. The control groups were bled and sacrificed in the same way as the infected groups.

Indirect systolic blood pressure (SBP) and heart rate (HR) measurements in conscious mice

Body weight and tail SBP were recorded weekly. SBP was measured by means of tail-cuff plethysmography (mod 8006; U.

Table 1
Western blot analysis in i.c. infected mice (values are the percentage of the density of the specific PrP^{res} signal produced by the reference sample (M ± SE).

	60 days	100 days	120 days
Brain	73.7 ± 6.8	85.8 ± 6.9	118.4 ± 8.6
Spleen	42.5 ± 7.8	48.9 ± 8.1	101.8 ± 8.1

Table 2
Histopathology scores of brains at different times after i.p. infection (M ± SD).

Days after infection	Histopathology scores (range 0–4)
50	0.17 ± 0.37
120	3.77 ± 0.40