PrP\textsuperscript{CWD} in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease

Christina J. Sigurdson, 1 Terry R. Spraker, 1, 2 Michael W. Miller, 3 Bruno Oesch 4 and Edward A. Hoover 1

1 Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1671, USA
2 Colorado State University Veterinary Diagnostic Laboratory, 300 West Drake Road, Fort Collins, CO 80523-1671, USA
3 Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, CO 80526-2097, USA
4 Prionics AG, University of Zürich 44J30, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Accumulated evidence in experimental and natural prion disease systems supports a neural route of infectious prion spread from peripheral sites of entry to the central nervous system. However, little is known about prion trafficking routes in cervids with a naturally occurring prion disease known as chronic wasting disease (CWD). In the brain, the pathogenic isoform of the prion protein (PrP\textsuperscript{CWD}) accumulates initially in the dorsal motor nucleus of the vagus nerve. To assess whether alimentary-associated neural pathways may play a role in prion trafficking, neural and endocrine tissues from mule deer naturally infected with CWD (n = 6) were examined by immunohistochemistry. PrP\textsuperscript{CWD} was detected in the myenteric plexus, vagosympathetic trunk, nodose ganglion, pituitary, adrenal medulla and pancreatic islets. No to scant PrP\textsuperscript{CWD} staining was detected in other nerves or ganglia (brachial plexus, sciatic nerve, gasserian ganglion, coeliac ganglion, cranial cervical ganglion, spinal nerve roots) of CWD-positive deer and no PrP\textsuperscript{CWD} was detected in nerves or endocrine tissues from 11 control deer. These findings suggest that: (i) transit of PrP\textsuperscript{CWD} in nerves, either centrifugally or centripetally, is one route of prion trafficking and organ invasion and (ii) endocrine organs may also be targets for cervid pathogenic prion accumulation.

Introduction

Chronic wasting disease (CWD) is an endemic transmissible spongiform encephalopathy (TSE) of captive and free-ranging mule deer, white-tailed deer and Rocky Mountain elk in Colorado and Wyoming (Williams & Young, 1980, 1982; Spraker et al., 1997). CWD is transmitted efficiently in nature, with the prevalence reaching 15% in some subpopulations (Miller et al., 2000). Relatively little is known, however, about the mode of transmission or the pathogenesis of this naturally occurring TSE. TSEs are characterized by the accumulation of a pathogenic, partially protease-resistant isoform (PrP\textsuperscript{res}) of a normal cellular protein (PrP\textsuperscript{c}) (Prusiner, 1982). PrP\textsuperscript{res} deposition occurs principally in the central nervous system (CNS), resulting in a neurodegenerative disease and in some prion infections, e.g. scrapie and variant Creutzfeldt–Jakob disease (vCJD). PrP\textsuperscript{res} deposition or infectivity also occurs in lymphoid tissues (Hadlow et al., 1982; Hill et al., 1999; Brown et al., 1999). Understanding routes of agent spread from peripheral entry sites to the CNS is fundamental to developing strategies to block neuroinvasion.

A growing body of evidence including both experimental and natural studies strongly implicates PrP\textsuperscript{res} dissemination from the entry site via peripheral nerves. Pioneering studies by Kimberlin and colleagues in intrasciatically or intragastrically inoculated mice demonstrated scrapie transport from the peripheral nervous system (PNS) directly to the brain or from the intestinal tract to the thoracic spinal cord via splanchnic nerves (Kimberlin et al., 1983; Kimberlin & Walker, 1989). Additionally, in hamsters challenged orally with 263K scrapie, PrP\textsuperscript{res} was detected by immunohistochemistry (IHC) in early infection within the myenteric and submucosal plexuses (Beekes & McBride, 2000) as well as the dorsal motor nucleus of the vagus nerve (DMNV) in the brain (Beekes et al., 1998) and terminally in the vagus nerve and the nodose, dorsal root...
and coeliac ganglia, findings consistent with spread via vagal and splanchnic routes (McBride & Beekes, 1999). van Keulen et al. (1999, 2000) demonstrated PrP^{RES} deposits in the enteric nervous system in natural sheep scrapie, which supported the alimentary tract as the site of neural invasion. Moreover, nerve infectivity or PrP^{RES} has been detected in natural (Hadlow et al., 1982; Groschup et al., 1996) and experimental (Groschup et al., 1999) scrapie, BSE-infected leums (Bons et al., 1999) and in a natural case of CJD (Hainfellner & Budka, 1999).

Recent findings have highlighted the significance of spread via peripheral nerves. Glatzel & Aguzzi (2000) compared PrP^{RES} tissue distribution patterns in wild-type versus transgenic PrP^{RES}-overexpressing mice. They demonstrated that elevated PrP expression in the PNS biased PrP^{RES} transit pathways toward intraneurval spread in transgenic mice versus lymphoreticular spread in wild-type mice. Race et al. (2000) demonstrated that transgenic mice expressing hamster PrP^{RES} in neural but not lymphoid tissues developed brain PrP^{RES} infection after oral or intraperitoneal inoculation with hamster scrapie, establishing a vital role for PrP^{RES} peripheral nerve expression in neuroinvasion.

As with other prion diseases (scrapie, BSE, kuru and vCJD), CWD infections are suspected to arise from oral exposure to the causative agent. In deer infected orally with brain containing PrP^{RES} (PrP^{CWD}), PrP^{CWD} deposition was first detected in alimentary-associated lymphoid tissues (Sigurdson et al., 1999) and subsequently in the DMNV in the medulla oblongata (Williams & Miller, 2000). In naturally infected deer, histological lesions of TSE and PrP^{RES} were present in the hypothalamus, thalamus, brainstem and spinal cord grey matter with smaller amounts in the cerebral cortex and cerebellum (Spraker et al., 1997, 2001). PrP^{RES} correlates closely with infectivity and serves as a surrogate marker for prion infection (McKinley et al., 1983; Race et al., 1998). Thus, IHC can be used to localize PrP^{RES} in tissues.

Because the DMNV is the initial target site for PrP^{CWD} in the brain (Williams & Miller, 2000) and the vago-sympathetic trunk carries vagal nerve fibres that innervate the alimentary tract, we examined alimentary nerves and ganglia from mule deer with naturally occurring CWD by using IHC. For comparison with non-alimentary nerves, we examined the brachial plexus and the sciatic nerve, which respectively innervate the forelimb and hindlimb. In order to investigate components of the sympathetic splanchnic circuity, coeliac ganglia and thoracic spinal cord with associated nerve roots were assessed. Gasserian ganglia were included to explore potential PrP transit via the trigeminal nerve. We report that the myenteric plexus, vago-sympathetic trunk and, to a lesser degree, the other peripheral nerves of deer contain PrP^{CWD}, indicating that nerve transport may be one route of PrP trafficking in CWD. An unexpected finding was the detection of PrP^{CWD} in pancreatic islet cells, adrenal medulla and the pituitary, suggesting nerve-vecorted transit may also occur to endocrine organs.

**Methods**

- **CWD-infected deer and tissue collection.** Six captive mule deer (Odocoileus hemionus) with naturally occurring, clinical CWD were euthanized and the following tissues were collected for assessment by IHC: brain, ~15 cm of the cervical vago-sympathetic trunk, 15–20 cm of the sympathetic trunk from the thoracic vertebral region, 8–10 cm of the sciatic nerve, 10 cm of the brachial plexus, a 4 cm^2_ segment of pancreas, the pituitary, adrenal gland, small intestine, coeliac and gasserian ganglia and thoracic spinal cord (Fig. 1). Tissues were fixed in 10% neutral-buffered formalin for 1–3 days and then immersed in 88% formic acid for 1 h and embedded in paraffin.

- **Negative-control deer and tissues.** Brain and vago-sympathetic trunk from eight free-ranging mule deer from a CWD non-endemic area (non-endemic area established by methods of Miller et al., 2000) and brain, sciatic nerve, adrenal gland, pancreas, small intestine, coeliac and gasserian ganglia, thoracic spinal cord and pituitary of three mule deer inoculated with CWD-negative brain homogenate from a previous study (Sigurdson et al., 1999) were similarly fixed and processed.

- **IHC staining.** Tissue sections were mounted onto positively charged glass slides, deparaffinized, hydrated, autoclaved in a citrate buffer solution (DAKO Target Antigen Retrieval) for 20 min at 121 °C and cooled for 5 min.

The IHC protocol employed an automated immunostainer (Ventana Medical Systems) and anti-PrP MAb 6H4 or 99/97.6.1 (generously provided by K. O’Rourke), a biotinylated secondary antibody, an alkaline phosphatase–streptavidin conjugate, a substrate chromogen (fast red A) and a haematoxylin and bluing counterstain (Ventana Medical Systems). MAB 6H4 recognizes a conserved sequence of the prion protein, corresponding to residues 144–152 of the human amino acid sequence.

![Fig. 1. Distribution of sympathetic, parasympathetic, cranial and motor nerves examined for the pathogenic isoform of the prion protein. Arrows indicate sites sampled. Number of stars indicates the incidence of PrP^{CWD} IHC positivity in the six deer, defined as the percentage positive of the total for each tissue sampled (0, 0%; *, 1–50%; **, 51–75%; ***, 75–100%). Abbreviations: b, brain; gg, gasserian ganglion; pi, pituitary; vs, vago-sympathetic trunk; n, nodose ganglion; cr, cranial cervical ganglion; bp, brachial plexus; sy, sympathetic trunk; sp, spinal cord; a, adrenal; c, coeliac ganglion; sc, sciatic; p, pancreas; i, intestine/myenteric plexus.](Image)
Fig. 2. IHC detection of PrP<sub>CWD</sub> in the central and peripheral nervous system of naturally infected mule deer using MAb 6H4. (a) Brain at medulla oblongata. PrP<sub>CWD</sub> stain in the DMNV (arrow), vagal radix (arrowhead) and presumably the vagus nerve exiting the section (inset). (b)–(e) PrP<sub>CWD</sub> labelling was detected in the vagosympathetic trunk (b, arrows) and in the myenteric plexus of the small intestine (d, arrowheads) of CWD-infected deer, but not in the control CWD-negative deer (c, e). Bars, 1 mm (a) or 10 μm (b, d).

and recognizes PrP epitopes of rabbit, mink, sheep, cattle and primates (Korth et al., 1997). MAb 99/97.6.1 recognizes residues 220–225 of the ovine prion protein (O’Rourke et al., 2000). An isotype-matched, irrelevant antibody was substituted in the IHC protocol as a negative control. Each immunostained nerve section was examined once or twice with each antibody.
Results

PrP<sub>CWD</sub> in nerve and ganglia

Given that alimentary exposure is likely in CWD, we focused on two major autonomic nerve tracts as potential PrP<sub>CWD</sub> conduits: (i) the vagosympathetic trunk and (ii) the splanchnic neural circuitry. The vagosympathetic trunk includes parasympathetic vagal nerve fibres, which have nerve cell bodies in the DMNV, pass through the nodose ganglion and synapse with the myenteric plexus of the small intestine.

The splanchnic nerves, which have nerve cell bodies in the intermediolateral cell column of the thoracic spinal cord, carry sympathetic fibres that synapse directly in the adrenal medulla or synapse in the coeliac ganglion and innervate the oesophagus, stomach and small intestine (Fig. 1).

CWD-positive deer were diagnosed by: (i) histological lesions of CWD in the medulla oblongata including perikaryonic neuronal vacuoles, spongiform degeneration of the neuropil and astrocytosis, and (ii) abundant PrP<sub>CWD</sub> stain in the DMNV by IHC. Deer were confirmed as CWD-negative by

---

Table 1. IHC detection of PrP<sub>CWD</sub> in nerves from CWD-infected mule deer

MAbs 6H4 and 99/97.6.1 were used to detect the prion protein. IHC stain was quantified as the number of positive stain granules in a section: +, < 10; ++, 10–20; ++++, > 20. ND, Not done.

<table>
<thead>
<tr>
<th>Deer case number</th>
<th>Tissue</th>
<th>MAb</th>
<th>V92</th>
<th>Za93</th>
<th>B93</th>
<th>Mb97</th>
<th>W97</th>
<th>H92</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vagosympathetic trunk</td>
<td>6H4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Vagosympathetic trunk</td>
<td>99/97.6.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Sciatic nerve</td>
<td>6H4</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Sciatic nerve</td>
<td>99/97.6.1</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Sympathetic trunk</td>
<td>6H4</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Sympathetic trunk</td>
<td>99/97.6.1</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Brachial plexus</td>
<td>6H4</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Brachial plexus</td>
<td>99/97.6.1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>25</td>
</tr>
</tbody>
</table>

—

Table 2. IHC detection of PrP<sub>CWD</sub> in neural and endocrine tissues from CWD-infected mule deer

MAbs 6H4 and 99/97.6.1 were used to detect the prion protein. ND, Not done.

<table>
<thead>
<tr>
<th>Deer case number</th>
<th>Tissue</th>
<th>MAb</th>
<th>V92</th>
<th>Za93</th>
<th>B93</th>
<th>Mb97</th>
<th>W97</th>
<th>H92</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medulla oblongata</td>
<td>6H4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Medulla oblongata</td>
<td>99/97.6.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Intermediolateral cell column of spinal cord</td>
<td>99/97.6.1</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Pituitary</td>
<td>6H4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Pituitary</td>
<td>99/97.6.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>6H4</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>99/97.6.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Adrenal medulla</td>
<td>99/97.6.1</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Myenteric plexus</td>
<td>99/97.6.1</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Coeliac ganglion</td>
<td>99/97.6.1</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nodose ganglion</td>
<td>99/97.6.1</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Cranial cervical ganglion</td>
<td>99/97.6.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gasserian ganglion</td>
<td>99/97.6.1</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spinal nerve roots, dorsal and ventral</td>
<td>99/97.6.1</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
</tbody>
</table>

* Associated nerve was negative.
the absence of histological lesions and negative staining for PrPCWD in all tissues. In one deer, abundant PrPCWD stain was present in the DMNV, the radix tract and the vagus nerve exiting the obex (Fig. 2a). PrPCWD was detected in the vagsympathetic trunk (n = 6 deer), sciatic nerve (n = 1), sympathetic trunk (n = 1) and brachial plexus (n = 1) (Table 1). The stain deposits appeared as scattered coarse granules within the nerve fascicles and occasionally within axons (Fig. 2b). The staining detected in the sciatic nerve and brachial plexus was scant compared with the vagsympathetic trunk.
No stain deposits were visible in the nerves from CWD-negative deer from known non-endemic geographical regions using MAb 6H4 (Fig. 2c). Three stain granules were detected in one nerve of one negative-control deer using MAb 99/97.6.1; therefore only nerves with more than three stain granules were considered positive. PrPCWD was present in the myenteric plexus (n = 5, Fig. 2d), nodose ganglion (n = 2) and in the intermediolateral cell column of the thoracic spinal cord (n = 4), but not in the cranial cervical, coeliac or gasserian ganglia (Table 2). Stain deposits in the nodose ganglia appeared primarily along nerve fibres and in satellite cells with little to no stain within the ganglion cell body. However, coarse stain deposits were present in the myenteric plexus neurons (Fig. 2d).

Minor differences in tissue-staining positivity were observed between MAbs 6H4 and 99/97.6.1 (Tables 1 and 2). The IHC stain deposits in nerve were irregularly distributed and widely spaced along the fascicle. Stain was quantified in the nerve only by the number of red PrP stain granules present in a section and recorded as + (four to ten), ++ (ten to twenty) or +++ (more than twenty). Nerve tissues incubated with an irrelevant MAb were negative.

**PrPCWD deposits in pituitary, islets of Langerhans and adrenal medulla**

The pancreases of five of six CWD-positive deer contained diffuse, coarse granular PrPCWD deposits confined to the islets of Langerhans (Fig. 3a). Although fewer than half of the islets were affected in any pancreas section in four of five deer, PrPCWD stain was abundant in the affected islets. Such deposits were not detected in pancreases of CWD-negative deer (Fig. 3b). In the pituitaries of all CWD-positive deer, PrPCWD deposits were evident, primarily in the pars nervosa and intermedia (Fig. 3c), and were not seen in the CWD-negative deer (Fig. 3d). Likewise, PrPCWD staining was identified in the adrenal medulla in three of five CWD-positive deer and not the controls.

**Discussion**

We detected PrPCWD in the myenteric plexus, vagosympathetic trunk and intermediolateral cell column of the spinal cord of naturally infected CWD deer, consistent with previous findings in experimental and natural TSE. Likewise, scrapie PrPres has been demonstrated in submucosal and myenteric plexuses in orally inoculated hamsters (Beekes & McBride, 2000) and in naturally infected sheep (van Keulen et al., 2000). These findings suggest that prion trafficking may occur by centripetal or centrifugal nerve transport. In orally challenged deer euthanized sequentially from 3–28 months post-inoculation (n = 20), PrPCWD was detected initially within the DMNV of the brain by IHC (Williams & Miller, 2000). The initial appearance of PrPCWD in the DMNV implicates the vagus nerve as a potential route for PrPCWD transit from the presumed site of exposure in the alimentary tract to the CNS.

Abundant PrPCWD was detected in the vagosympathetic trunk and in nerve fibres in the nodose ganglion, compared with scant or no deposition of PrP in the cranial cervical ganglion (sensory), coeliac ganglion, sciatic nerve or brachial plexus, which suggests that the vagus nerve could serve as a major transit route of PrPCWD. PrPCWD was detected in myenteric ganglion cell bodies, along nerve fibres and in satellite cells, as has been described in other studies (Groschup et al., 1999; McBride & Beekes, 1999). Nevertheless, other routes of PrPCWD transit, such as via blood, sensory or cranial nerves innervating the oral mucosa (IX, X) or sympathetic splanchnic nerves, cannot be excluded. In haematogenous dissemination, it might be expected that PrPCWD amplification would occur initially in richly vascular neural domains with fenestrated endothelium (e.g. area postrema of the medulla oblongata, hypophysis, pineal body, hypothalamic regions, subfornical organ) as opposed to the DMNV. Dissemination via cranial nerves IX and X might be expected to result in initial PrPCWD amplification in the nucleus solitarius.

The gastrointestinal tract receives parasympathetic vagal nerve fibres from the DMNV and sympathetic nerve fibres from the spinal cord via the coeliac ganglion (splanchnic circuitry). In the light of PrPCWD detection in the intermediolateral column of the spinal cord and adrenal medulla, which is innervated by splanchnic nerves, it is plausible that PrPCWD may also traffic to the CNS via the splanchnic circuitry. If this is the case, it is surprising that we did not detect PrPCWD in the coeliac ganglion, in which pre-ganglionic splanchnic nerve fibres to the intestine synapse. It is possible that PrPCWD in the intermediolateral column resulted from spread within the CNS and then spread centrifugally to the adrenal medulla, a route that does not involve the coeliac ganglion.

Neurotropic viruses that enter the host via the gastrointestinal tract have been investigated to determine their route of entry into the CNS. The pathogenesis of prion infections has been compared to that of pseudorabies virus (Beekes et al., 1998), which spreads retrograde along parasympathetic vagal efferents to the DMNV (Card et al., 1990). Similarly, in mice inoculated perorally with a neurotropic reovirus, the virus spread to the myenteric plexus and then retrograde along the vagus efferent nerve to the DMNV, regardless of the amount of virus in the bloodstream. Moreover, subcutaneous inoculation over the forehead resulted in the detection of virus in the facial and trigeminal nuclei of the brain, but not in the DMNV, establishing that detection of virus in the DMNV depended on an oral inoculation route (Morrison et al., 1991).

Substantial deposits of PrPCWD were detected in the pancreatic islet cells, which are innervated by the vagus nerve (Loewy et al., 1994). PrP-containing islets were often adjacent, which might suggest infection from a common nidus, such as...
innervation by a common nerve branch. Infectivity in the pancreas has been documented previously in natural and experimental scrapie infections (Pattison & Millson, 1960; Ye et al., 1994b) and PrP has also been demonstrated in islets from uninfected and scrapie-infected mice (McBride et al., 1992). Alterations in islet function have not been examined, although hamsters infected with the 139H scrapie strain develop obesity and hypoglycaemia/hyperinsulinaemia with extensive pituitary and pancreatic vacuolation (Ye et al., 1994a; Ye & Carp, 1996). Pancreatic lesions were localized to islet beta cells, amyloid deposits were not found and scrapie infectivity was extremely low (Ye et al., 1997).

The PrP<sub>CWD</sub> detected in the adrenal medulla could be derived from PrP transport via the splanchnic nerves arising from nerve cell bodies in the intermediolateral column of the spinal cord, which has demonstrable PrP<sub>CWD</sub>. We also demonstrated PrP<sub>CWD</sub> in the pars intermedia and nervosa of the pituitary in CWD-infected deer. Potentially, PrP<sub>CWD</sub> could transit via nerve fibres from the hypothalamus to the pars nervosa, as deer with CWD have abundant PrP<sub>CWD</sub> deposition in the hypothalamus (Spraker et al., 2001). Histological lesions were not evident in either the adrenal or pituitary; however, it is not known whether functional disturbances such as altered hormone synthesis are associated with PrP<sub>CWD</sub> deposition.

In summary, the findings reported here in CWD-infected deer provide circumstantial evidence for: (i) trafficking of PrP<sub>CWD</sub> in the peripheral nerves and (ii) localization of the pathogenic prion protein in the endocrine system of CWD-infected deer. These observations are consistent with assembled findings in other experimental and natural TSEs and may lend insight into the potential pathways of prion trafficking in cervid CWD.

We are grateful to Katherine O’Rourke for providing MAb 99/97.6.1. We thank Margaret Wild, Kate Larsen, Caroline Krumm, Erin Meyers and Sam Hendrix for assistance with tissue collection. We appreciate the excellent histotechnology support of Robert Zink and Bruce Cummings and gratefully acknowledge Drs Daniel Gould and Elizabeth Williams for critical review of the manuscript. This work was supported by grants from the Colorado Division of Wildlife, the College of Veterinary Medicine and Biomedical Sciences Research Council, Colorado State University and NIH RO1-Al49171. C. Sigurdson was supported by a fellowship from the USDA, 97-36200-5238, and by NIH K08-Al01802-01.

References


Received 8 May 2001; Accepted 16 July 2001