Acta Neuropathol (1996) 92 : 409–414

SHORT ORIGINAL COMMUNICATION

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Degenerative axonal swellings in the trigeminal ganglia of cattle

Received: 4 December 1995 / Revised: 16 January 1996 / Revised, accepted: 22 April 1996

Abstract We examined the acidophilic oval structures which were frequently encountered in the trigeminal ganglia of cattle that clinically showed trigeminal disturbance. Histopathologically, these structures were composed of numerous fine granules and were generally located near the neuronal cell bodies. They were demonstrated in silver preparations and, using neuron-specific enolase, tau and ubiquitin antibodies. Electron microscopy revealed an accumulation of large membranous vesicles thought to be derived from the smooth endoplasmic reticulum of axons, a few degenerative mitochondria and non-membrane-bound osmiophilic debris. Such pathological alterations were consistent with degenerative axonal changes or enlargements, although the pathogenesis was not clear. It should be emphasized that such axonal degeneration is frequently observed as a nonspecific reaction in the trigeminal ganglia in various diseases that cause trigeminal failure in cattle.

Key words Axonal degeneration · Smooth endoplasmic reticulum · Trigeminal neuropathy · Trigeminal ganglia · Cattle

Introduction

The phenomenon of axonal swellings has been observed in a great variety of pathological conditions including trauma, tumors, vascular lesions, degenerative and demyelinating processes, and generalized metabolic disturbances [2, 4]. Axons react to injury by enlargement at the site of damage or at a certain distance from it. Such reactive or degenerative axonal enlargements are thought to be secondary or nonspecific reactions to a disturbance in the surrounding tissue [2]. The characteristic features seen in reactive, degenerating and regenerating axonal alterations can be differentiated histochemically and electron microscopically from a type of axonal enlargement referred to as axonal dystrophy or spheroids [4].

We examined the trigeminal ganglia of cattle showing the clinical symptoms expected in disturbances with trigeminal nerve involvement, although the causes or pathogenesis were varied. Degenerative axonal swellings were observed in these trigeminal ganglia but showed individual variations in number. Similar structures have been reported briefly in the trigeminal ganglia of the rhesus monkey [5]; however, such alterations appear to be uncommon in the trigeminal ganglia of most mammals. In this report, we describe the morphology of these structures observed in the trigeminal ganglia of the cattle.

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Materials and methods

Five cows (cases 1–5), 1–18 years old, were used in this study. Individual cases were afflicted with various diseases diagnosed following clinical and pathological examinations (Table 1). Two cows, 4 and 10 years old, without clinical neuromuscular disease were used as controls for the histopathological and electron microscopy studies.

Samples collected from the central nervous system (CNS), including trigeminal ganglia and spinal cord, and from visceral organs, were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned serially at 4 mm. Sections were stained with hematoxylin-eosin. Selected sections of the CNS and trigeminal ganglia were stained by periodic acid-Schiff, phosphotungstic acid hematoxylin, azan, Klüver-Barrera's and Bielschowsky's (modified by Hirano) methods. For immunohistochemistry, dewaxed sections were submitted to the avidin-biotin-peroxidase complex (ABC) method using an ABC kit (Vector Laboratories, USA). The antibodies used in this study were monoclonal anti-tau (1:400; Sigma, USA), polyclonal anti-ubiquitin (1:800; Dako, Denmark), polyclonal anti-neuron-specific enolase (NSE; 1:1000, Dakopatts, USA) and monoclonal anti-human neurofilament protein (1:400; Dako).

For electron microscopy, small samples of the trigeminal ganglia were immersed in 4% paraformaldehyde in 0.5% phosphate buffer, post-fixed in 1% osmium tetroxide, embedded in resin and processed routinely for semithin and ultrathin sectioning.

Table 1 Summary of the present cases (+ few, ++ moderate, +++ many, *N.E.* not examined, *IBR* infectious bovine rhinotracheitis)

Case no.	Age in years	Clinical or pathological diagnosis	Clinical symptoms				Axonal swellings in trigeminal ganglion
			Auricle hanging	Excess salivation	No corneal sensation	Facial analgesia	
1	1	Idiopathic trigeminal paralysis	Yes	Yes	Yes	Yes	+++
2	3	Hydrocephaly	Yes	Yes	Yes	Yes	++
3	3	Listeric encephalitis and trigeminal ganglioneuritis	Yes	Yes	N.E.	Yes	++
4	4	Trigeminal ganglioneuritis in IBR	No	Yes	N.E.	N.E.	+
5	18	Metastatic tumor in the trigeminal ganglion	No	Yes	N.E.	N.E.	++

Results

Clinical findings

Clinical symptoms are summarized in Table 1. No abnormalities in gait, excretion, or other general conditions including blood and serum examination were seen in any of the cases.

Cases 1–3 showed mouth and auricle hanging, and difficulty in eating and drinking. These cows experienced a lowering of pain and tactile sensation over the whole trigeminal field, especially in the nasal region. The motor function of the fifth cranial nerve, including mastication and swallowing, was preserved. Movement of the eyeballs was also normal. The signs had started suddenly and there had been no visible improvement in condition. Case 4 showed excess salivation and nasal flow. Infectious bovine rhinotracheitis (IBR) virus was isolated from the nasal flow, and a clinical diagnosis of respiratory-type IBR was made. In case 5 a relapse, due to a neoplasm, occurred 7 months after surgical removal of the primary tumor in the right eyelid, at which time a diagnosis of squamous cell carcinoma was made. Although there was tumor infiltration into the orbit, clinical signs expanding into the cranial cavity were not observed. Clinical examinations of trigeminal sensation were not conducted in cases 4 and 5.

Anatomical findings

In cases 1 and 4, no abnormalities were observed in the nervous system. Superficially, the cerebrum of case 2 showed swelling and softening on palpation. Elevation of the ventricular cerebrospinal fluid caused dilation of the lateral ventricle and expansion of the telencephalon. Malformation or obstruction of the mesencephalic aqueduct was observed and was diagnosed as obstructive hydrocephalus. The brain stem, cerebellum and trigeminal ganglion were normal. In case 3, a small malacic softening was seen in the pons and medulla oblongata, and final differentiation was confirmed by the isolation of *Listeria monocytogenes* from the malacic area. In case 5, neoplasm had metastasized to the submaxillary and parotid glands, and adjacent lymph nodes. The neoplasm also extended into the base of the cranial cavity through the orbit. None of the cases showed any abnormalities related to the clinical symptoms in the visceral organs.

Light microscopy findings

The trigeminal ganglia of the control animals appeared normal except for mild interstitial cellular infiltrations and a scattering of residual nodules of Nageotte (Fig. 1a).

The features common to the trigeminal ganglia in all affected cases were a number of membrane-bound acidophilic oval structures and several satellite cells (Fig. 1b–d), although their frequency showed individual variations (Table 1). The oval structures were composed of numerous fine granules and were generally located near the neuronal cell bodies (Fig. 1c, d), which they appeared to compress (Fig. 1b, c). In case 1 these structures accumulated around the cell bodies (Fig. 1b). In mild to moderate cases (nos. 2–5), they were solitary and sparsely distributed, and were often enclosed by several satellite cells (Fig. 1d). No histological continuity could be demonstrated among these structures, or between them and the neuronal cell bodies or processes. These structures also showed a granular brown staining using Bielschowsky's method. Immunohistochemically, strong but granular staining was demonstrated with ubiquitin, NSE and tau antibodies (Fig. 1e). However, neurofilament antigen was very sparse in these structures. Another common feature was an increase in the number of residual nodules of Nageotte, although statistical analysis was not done (Fig. 1f). In cases 3 and 4, moderately diffuse or nodular infiltration by lymphocytes and macrophages was the most conspicuous histological abnormality. Metastatic carcinoma in the perineurium of the trigeminal ganglia was observed in case 5. No abnormalities were seen in the trigeminal sensory roots of any of the cases.

In case 1, no abnormalities other than a few spheroids in the dorsal nuclei of the medulla oblongata were observed in the CNS, and the pathogenesis could not been determined. Case 2 revealed calcification in the choroid plexus of the lateral ventricule, and edema and severe gliosis in the nervous tissue along the lateral ventricule. In case 3, focal malacic lesions and macrophage nodules were observed in the reticular nuclei of the medulla oblongata, and in the corticospinal tract and dorsal part of the pons. Diffuse infiltration by lymphocytic cells and

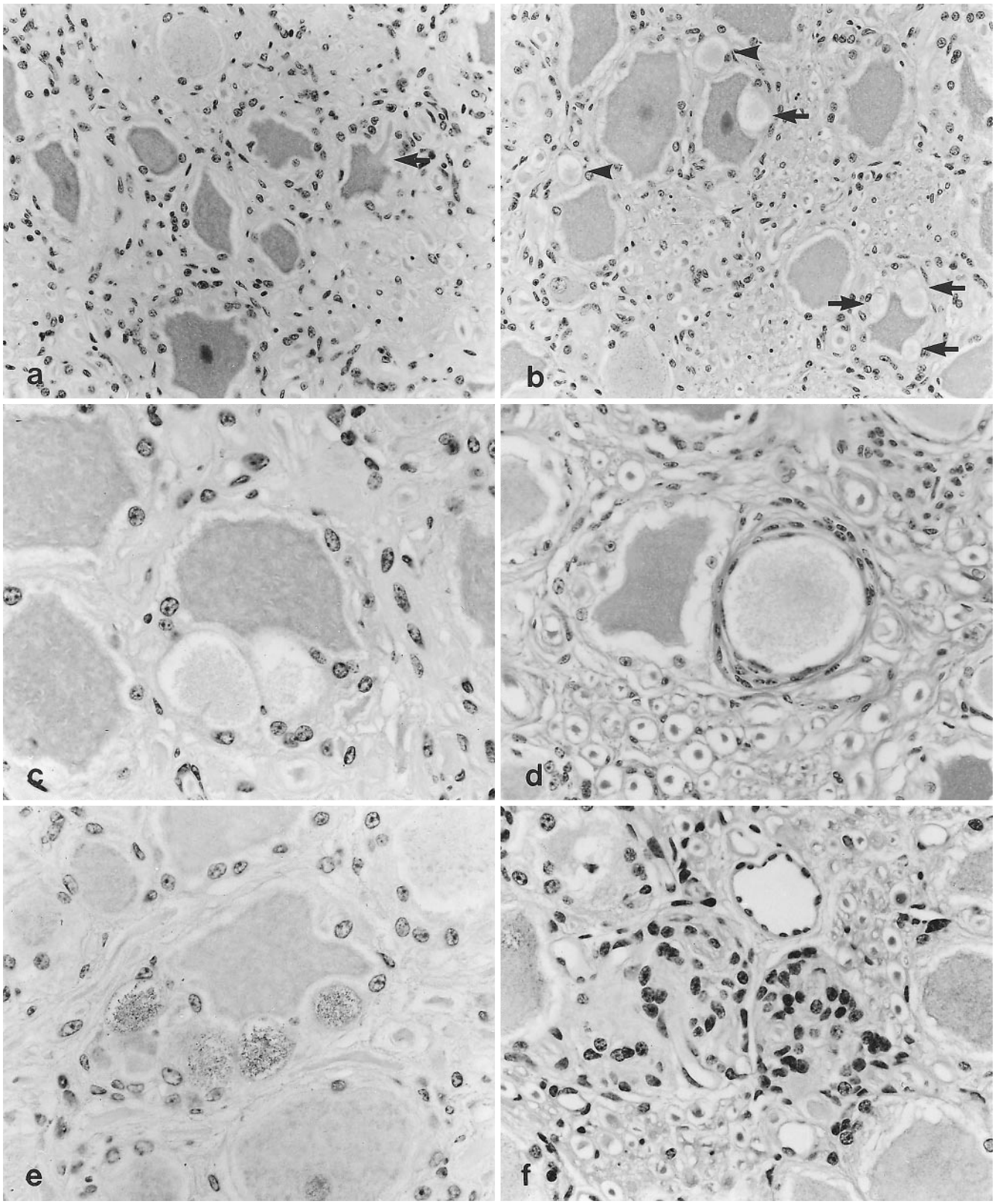

Fig. 1a–f Light micrographs of trigeminal ganglia. **a** A 4-year-old control animal. A nerve cell gives rise to a fine axon (*arrow*). **b** Case 1. A few acidophilic oval structures are present. Some (*arrows*) are located around the nerve cells. Others (*arrowheads*) are seen scattered among nerve cells. **c** Case 2. Higher magnification of the structures reveals fine granular contents bounded by a membrane. **d** Case 5. The structures can be seen enclosed by several satellite cells. **e** Case 1. The structures show granular materials with intense immunoreactivity for ubiquitin. **f** Case 1. Nodules of Nageotte are present among nerve cells. **a–d**, **f** H&E, **e** avidin-biotin-peroxidase complex method with anti-ubiquitin antibodies. **a**, **b** × 240, **c** × 500, **d** × 320, **e** × 470, **f** × 400

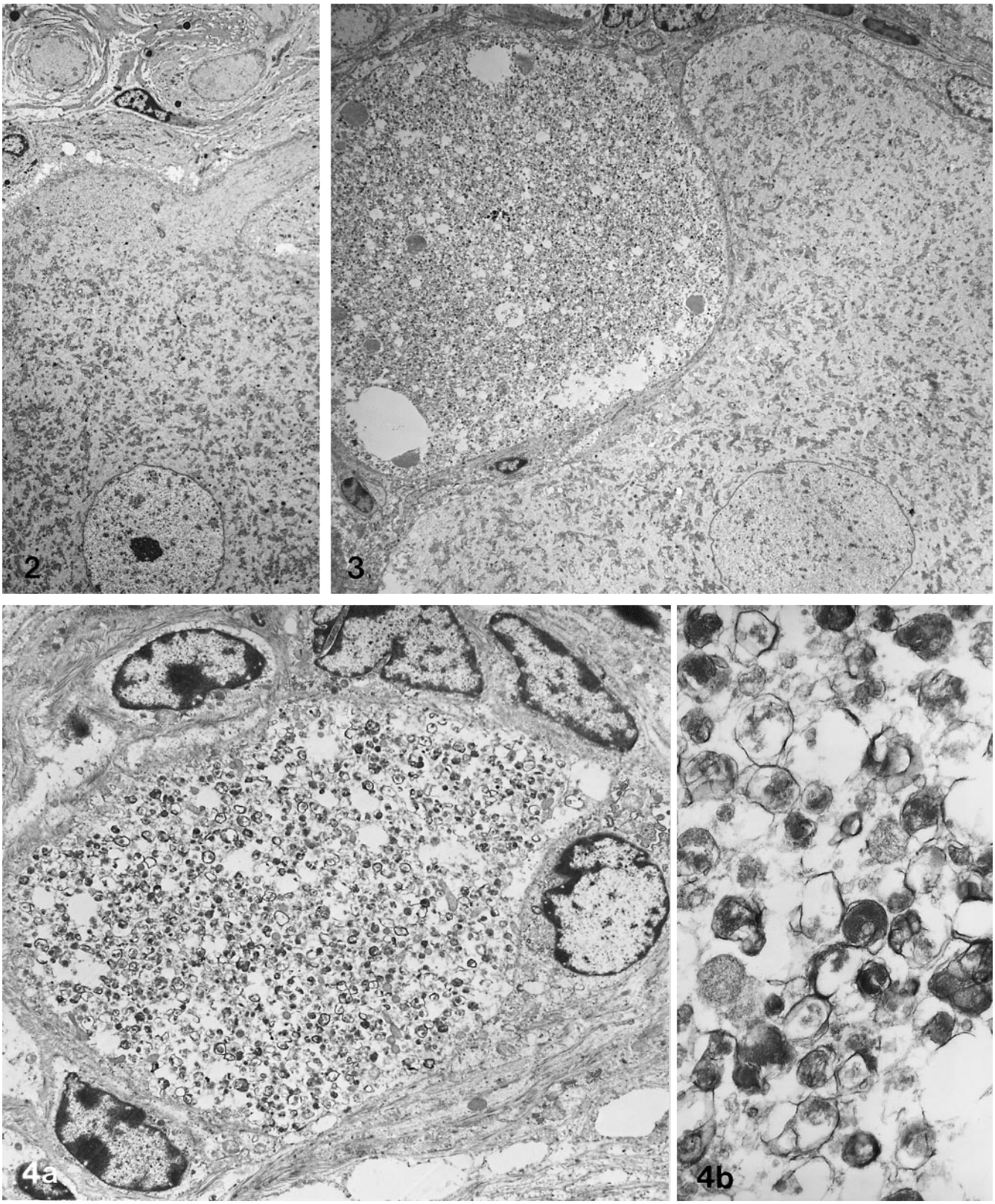

Fig. 2 Electron micrographs of a trigeminal ganglion from a 10-year-old control animal. An unmyelinated axon extends from axon hillock almost devoid of Nissl substance. The two unmyelinated axons are probably the initial portion of the same cell process. × 1500

Fig. 3 Electron micrographs of a trigeminal ganglion from case 1. An accumulation of dense granular materials and osmiophilic debris lies in contact with the nerve cell. No membrane boundary surrounding the abnormal structure is present. × 1500

Fig. 4a, **b** Electron micrographs of a trigeminal ganglion from case 3. **a** An accumulation of vesicular structures is surrounded by several satellite cells and collagen fibers. **b** Higher magnification shows an accumulation of membranous vesicles. **a** × 4200, **b** × 25000

perivascular cuffing was also seen in the brain stem. Case 4 showed mild perivascular cuffing and small glia nodules in the medulla oblongata and pons. Case 5 showed no abnormalities except for age-related changes such as spheroids and polyglucosan bodies in the dorsal funiculus of the medulla oblongata, and lipofuscin pigmentation of neuronal cell bodies.

Electron microscopy findings

The neurons in the control animals were invested by the cytoplasmic processes of surrounding satellite cells, and also gave rise to an axon (Fig. 2). In addition, unmyelinated axons surrounded by Schwann cells were seen near the neuronal cell body, which was thought to be the initial portion of the neuronal cell body.

Electron microscopy of the acidophilic oval structures revealed an accumulation of dense granular material and osmiophilic debris without a clear membrane (Fig. 3). These structures lay in close contact with the neuronal cell bodies, but were bounded by the unit membrane of the nerve cell and the satellite cell processes. Moreover, the oval structures were occasionally seen free from the neuronal cell bodies, which were surrounded by collagen fibers and several satellite cells (Fig. 4a). A more detailed examination showed that these structures were composed of large membranous vesicles and degenerative mitochondria, but no neurofilaments were demonstrated on electron microscopy (Fig. 4b).

Discussion

Axonal changes can be divided into reactive, degenerative, regenerative and dystrophic alterations [2, 4]. Among these alterations, the degenerative axons (called the retraction balls or granulated bodies) represent granular disintegration of neurofilaments and swollen mitochondria. The accumulated mitochondria change into membrane-bound, floccular, osmiophilic dense bodies in the enlarged, swollen proximal and distal stumps [4]. In the present study, the staining characteristics of the acidophilic oval structures (showing positive reactions for NSE and neurofilament antigens, as well as silver staining) would appear to indicate that these degenerative structures arose from the neurons. In addition, the ultrastructural alterations consisting of large membranous vesicles, degenerative mitochondria, and dense bodies are consistent with the degenerative axonal changes, although neurofilaments could not be demonstrated.

Degenerative axonal alterations have been observed under various experimental conditions of axonal transport interruption such as ischemia, crush injury or cooling [3, 9, 10]. According to those experiments, membranous vesicles are largely derived from the smooth endoplasmic reticulum (SER) associated with anterograde transport [9]. Because such morphological changes are caused by an interruption in transport due to local injury, it is likely that the restricted disorganization of the SER seen in this study [9] could be responsible for the focal stasis of axonal-transported protein. Furthermore, ubiquitin, which is a protein associated with ATP-dependent protease, combines specifically with degenerative proteins. In the study of Tsukita and Ishikawa [9], from the immunoreactive intensity and ultrastructural findings ubiquitin seemed to be present in the membranous vesicles, but this was not demonstrated convincingly. In the meantime, studies of ubiquitin immunoreactivity suggest that there is local activation of the ubiquitin system in an attempt to remove excessive and abnormal proteins [6].

Trigeminal neuropathy or trigeminal sensory neuropathy is defined as a clinical symptom or syndrome characterized by the loss of feeling or a prickling numbness in trigeminal nerve distribution [7]. Associated disorders include connective tissue disease, primary or metastatic tumor in the trigeminal nerve and inflammation caused by reactivation of a latent herpes simplex infection. However, pathological evidence of the site and nature of the lesion is either poor or completely lacking [7]. The axonal alterations reported here have not been described in the disorders showing trigeminal disturbance, although such alterations (referred to as retraction balls) are commonly observed in the nervous system as characteristic features in certain disorders, including diffuse axonal injury, periventricular leukomalacia and lysosomal storage diseases [1, 8].

The severity of case 1 might indicate close agreement with trigeminal failures or disturbance, whereas the other cases have been apparently regarded as reflections of the main pathological lesions including tumor, inflammation or defect. Although changes in chromatolytic or vacuolar degeneration were not observed, loss of neuronal cell bodies represented by residual nodules of Nageotte [1] was frequently encountered in all cases, which is in good agreement with those axonal alterations. Case 1 can be distinguished from the other four cases, because the etiology inducing the axonal degeneration in case 1 was uncertain and the alterations and clinical symptoms of the other four cases are thought to be obviously secondary. However, since pathological specificities and the nature of the trigeminal neuropathy have not been established as stated above, the present study does not deal with case 1 as an independent entity. This fact further suggests that the severity of axonal degeneration and subsequent neuronal cell loss reflect a decrease in trigeminal sensation, although the drooping of ears and lips in animals (cases 1–3) might be due to facial nerve involvement.

In this report, it should be emphasized that degenerative axonal swellings are frequently observed as nonspecific reactions in various diseases causing trigeminal failure in cattle, although how the degree of these alterations is related to the clinical symptoms should be examined.

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