Canine Neuroaxonal Dystrophy

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Abstract. Canine neuroaxonal dystrophy, a newly recognized familial disorder in Rottweiler dogs, is characterized by progressive sensory ataxia. Two of four dogs studied clinically were autopsied and the cerebellum was mildly atrophic. Massive numbers of axonal spheroids were present in many regions of the neuraxis but were most prominent in the dorsal horn of the spinal cord and the nuclei gracilis and cuneatus. Ultrastructurally, spheroids appeared to be swellings of distal axons which were filled with accumulations of smooth membrane-bound vesicles, membranous lamellae, dense bodies, and other organelles. Neuropathological changes were similar to those identified in human neuroaxonal dystrophy.

Key Words: Axons, dystrophic; Axons, transport; Dogs, Rottweiler; Neuroaxonal dystrophy; Spheroid, axonal.

INTRODUCTION

Membrane-filled swellings of distal axons are characteristic of a group of inherited human degenerative disorders, including infantile and juvenile neuroaxonal dystrophy (NAD) and Hallervorden-Spatz disease (HS) (1–15). Dystrophic changes in axons include distension of synaptic terminals by accumulations of smooth membranes, tubulovesicular elements, and mitochondria. Similar axonal changes have been recognized as a concomitant of aging and in human disorders in which absorption of fat-soluble vitamins is impaired (14, 16–21). Experimentally, such changes have been produced by vitamin E-deficient diets in laboratory animals (22–24) and by administration of toxins, such as tri-o-cresyl phosphate (25–29), zinc pyridinethione (ZPT) (30, 31), p-bromophenylacetylene (BPAU) (32–35), and diethyldithiocarbamate in sheep (36). The pathogenetic mechanisms underlying the development of this type of axonal abnormality are not well understood.

The kindred of Rottweiler dogs described in this report with a familial, progressive sensory ataxia characterized by distal axonal changes is of interest because so little is known of the pathogenesis of the inherited axonal dystrophies, and because other animal models of these genetic disorders are not available (37, 38). The syndrome, canine neuroaxonal dystrophy (CNAD), has clinical and pathological disease features in common with the human neurological disorders, NAD and HS, and should provide a useful animal model for investigating the pathogenesis of dystrophic axonal changes in heredodegenerative disease.

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MATERIALS AND METHODS

Four affected dogs (two females and two males) were examined, and pedigrees and clinical histories were reconstructed on the basis of information obtained from owners, breeders, and referring veterinarians. Autopsies were performed on a three-year-old female and a four-year-old male. Both dogs were heparinized and anesthetized with an overdose of intravenous sodium pentobarbital. One dog was perfused via the left ventricle with physiological saline, followed by a mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde, and subsequently by 4% paraformaldehyde and 6% glutaraldehyde in 0.1 M phosphate buffer. Tissues from the other dog were fixed by immersion in 10% neutral buffered formalin. Tissue samples of brain, spinal cord, viscera, and selected peripheral nerves and muscles were dehydrated through graded alcohols, embedded in paraffin, sectioned (7 μm), and stained with hematoxylin and eosin, Luxol fast blue (LFB), cresyl violet, periodic acid Schiff (PAS), and silver impregnation stains (Sevier-Munger) for examination by light microscopy. Selected regions of the central nervous system (CNS), peripheral nerves, and dorsal root ganglia were postfixed in osmium tetroxide, dehydrated, embedded in Epon, sectioned, mounted, and stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in an AEI 801 electron microscope.

RESULTS

Clinical Manifestations

Three of the four owners indicated that dogs were normal throughout the first year of life; the fourth owner said the dog was poorly coordinated and abnormally clumsy in the first year. All owners reported that at one year of age the dogs were unwilling or unable to jump over low obstacles. One owner reported that the dog appeared to have difficulty maintaining balance when standing in one place. At the time of examination, two dogs were four years old and the others were three years old and 19 months old. All were alert and appeared more playful than is usual for adult dogs. Two dogs (three and four years old) moved constantly, shifting their weight from one limb to the other when standing in one place. Although owners of the three- and four-year-old dogs reported decreased visual acuity, no abnormalities were noted on funduscopic examination of any dog, and all dogs followed visual stimuli well. Both four-year-old dogs had a horizontal nystagmus in the direction of gaze; no other cranial nerve abnormalities were recognized. Muscle bulk, tone, and strength were normal, and no involuntary movements were apparent. Deep reflexes were active and symmetrical. Nociception was unremarkable; proprioception was abnormal in all dogs. All dogs would stand with their feet in abnormal postures, e.g., with legs crossed or on three legs with one elevated (Fig. 1B). The 19-month-old and three-year-old dogs tended to knuckle over, particularly on the hind limbs, and drag their feet (Fig. 1A, B). All dogs had delayed placing reflexes.

The most striking clinical feature was a prancing, swinging gait that was more evident in the three- and four-year-old dogs. They walked with an enlarged base and dysmetria. The legs were lifted abnormally high with an excessively long stride. Misdirection of steps was more pronounced in the forelimbs of all animals. In the older dogs, the feet tended to cross over the midline, and all had difficulty negotiating turns. Difficulty in limb placement was most easily recognized in climbing stairs, where the range and force of movement could not be controlled (Fig. 1C, D). By four years of age, dogs were virtually unable to climb or descend stairs. Two of the three dogs had clinical signs of hip dysplasia (malformation of the coxofemoral articula-
Fig. 1. Two-year-old male Rottweiler dog (A) showing evidence of sensory impairment and loss of proprioception by dragging of front foot, and (B) peculiar stance with crossed forelegs. (C, D) Four-year-old female Rottweiler dog showing signs of cerebellar deficit indicated by inability to control the range of limb movement. There is excessive excursion of front leg (arrow) in descending steps.

tion), a common problem in breeds of large dogs. The four-year-old female had an acquired patchy depigmentation of the skin.

Laboratory Findings

Complete blood count was normal, and serum levels of electrolytes, urea nitrogen, creatinine, uric acid, glucose, albumin, globulin, bilirubin, cholesterol, triglycerides, lactic dehydrogenase, glutamyl transpeptidase, glutamic-oxalacetic transaminase, and alkaline phosphatase were unremarkable. Plasma and urine amino acid levels and serum vitamin E levels were also normal. Cerebrospinal fluid (CSF) was also examined in the 19-month-old dog and in the four-year-old dog. CSF protein was 40 mg% in the 19-month-old (normal range 0–20 mg%) and unremarkable in the older dog.

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Electromyography in three dogs detected fibrillation potentials compatible with denervation in the foot muscles but not at more proximal levels, a pattern seen frequently in neurologically intact dogs. Motor nerve conduction velocities were normal.

Genetic Features

The pedigree (Fig. 2) of this family shows the degree of inbreeding present in this kindred. Three of the affected dogs were sired by one male (Dog No. 2), and the fourth dog is the progeny of this sire’s female sibling (Dog No. 3). In a previous litter, she had produced a male dog with similar clinical signs. In these litters, two of the dams are sisters; all affected dogs share a common female ancestor (Dog No. 1), and both males and females are affected. An autosomal recessive mode of inheritance is suggested by the following features: affected dogs have clinically normal parents, and both males and females are affected.

Pathological Findings

Except for the presence of hip dysplasia, an acute bacterial prostatitis (E. coli), and chronic pyelonephritis in the four-year-old male, significant lesions were confined to the nervous system.

Neuropathological Findings

Macroscopic examination showed a mild atrophy of the cerebellum, more pronounced in the midline and surrounding the primary fissure. The optic nerves were small.

Light microscopic examination disclosed the presence of large numbers of axonal spheroids (up to 100 μm in diameter). In hematoxylin and eosin-stained sections, the appearance of the oval-to-circular spheroids was variable: some were densely eosinophilic and either smoothly homogenous or granular; others were palely vacuolated, slightly stippled profiles with central cores of swirling filaments or granules.

Fig. 2. Pedigree of Rottweilers with CNAD. Affected males and females are indicated by solid squares (males) and circles (females). Asterisks indicate affected dogs shown in Figure 1. Diamonds indicate that number or sex of littermates is unknown. Diagonal bar indicates dogs known to be dead. Female No. 1 is common ancestor to all dogs. Male No. 2 sired three affected dogs, and his sibling, No. 3, has produced two affected progeny.
(Fig. 3A). The central cores stained variably with PAS and LFB, and fibrillar and granular material within axons was frequently argyrophilic (Fig. 3B). A "bull’s-eye" appearance was commonly seen. In toluidine blue-stained 1-μm Epon sections, the vacuolated-to-granular appearance of the spheroids was enhanced, and the presence of a myelin sheath, often attenuated, was apparent surrounding many of these axons (Fig. 3C). Spheroids appeared at times to abut the perikaryon and dendrites of neurons, particularly in the ventral horn of the spinal cord. Neurons were not altered, although abundant glial processes frequently surrounded the neuronal margin. Small amounts of PAS-positive debris were present in macrophages adjacent to vessels. There was a mild loss of Purkinje and granule cells in the cerebellum which was more marked in the vermis. Autonomic ganglia within the abdomen were not remarkable. Sections of peripheral nerve trunks, distal peripheral nerves, and the neuromuscular junction which were examined were not remarkable. No evidence of abnormalities in the Pacinian corpuscles or other sensory terminals were detected in routine examinations.

Electron microscopic study of the spheroids revealed that they were enlarged axons and synaptic terminals. Swollen axons contained a variety of constituents: tubulovesicular profiles (Fig. 4A), smooth membrane (Fig. 4B, C), flat lamellae, and dense core vesicles (Fig. 4A). Vesicles might be lucent or contain granular or flocculent material. In longitudinal section, the dystrophic portion of the axon formed a bulbous distention at the presynaptic terminal, and the distended portion of the axon frequently contained a central channel of cytoskeletal elements, mostly neurofilaments (Fig. 4A). When seen in cross section, some of these swollen axons contained an intact, if somewhat attenuated, myelin sheath; in other sections, the myelin sheath was intact above the region of enlargement but absent around the spheroid itself (Fig. 4A).

Distribution of Spheroids

Massive numbers of spheroids almost replaced the neuropil in selected regions of the neuraxis (Fig. 3C). In the dorsal horn of the spinal cord, the majority of the swellings occupied the distal region of dorsal root afferent fibers, and the topography of the spheroids in other regions was consistent with a process affecting mostly sensory axon terminals. Spheroids were most numerous in the vestibular nucleus; in the lateral and medial geniculate bodies; in the sensory nucleus of the trigeminal nerve; in the gracilis, cuneatus, and accessory cuneatus nuclei; and in lamina I and II of the spinal cord. Spheroids were also present, but less numerous, in the inferior olive, trochlear, and oculomotor nuclei and in the ventral horn of the spinal cord. Spheroids were occasionally present in the globus pallidus, hippocampus, hypothalamus, thalamus, caudate, and reticular substance; they were not seen in the cerebral cortex. Within the cerebellum, spheroids were not present in the molecular layer but were seen within the granular layer, white matter, and deep nuclei. They were more frequent in the vermis and pars intermedia than in the hemispheres.

DISCUSSION

Inheritance of Neuroaxonal Dystrophy

CNAD is a newly recognized familial disorder in Rottweiler dogs, probably inherited as an autosomal recessive trait. A test mating of affected dogs has been made to
Fig. 3. A. Axonal spheroids in vestibular nucleus from a three-year-old female Rottweiler dog showing LFB-positive and filamentous material. Many axons within the nucleus are distended and contain particulate or filamentous material. LFB-PAS. ×610. B. Spheroid in the dorsal column of the lumbar cord from a three-year-old Rottweiler dog. The concentrically arranged swirls of argyrophilic filaments occupy the center of this distended axon. Sevier-Munger. ×400. C. Spheroids in the dorsal horn of the lumbar spinal cord in a four-year-old Rottweiler dog. There is variation in size and character of the spheroids which virtually replace the normal neuropil. Some spheroids retain portions of a myelin sheath (arrow); in others it was attenuated and frequently was absent. Within the spheroids, there is granular, filamentous, membranous, or vesicular material. Toluidine blue. ×810.
Fig. 4. A. This dorsal root sensory fiber contains a distended segment which is demyelinated over the enlarged region (top) nearer the spinal cord. Within the dystrophic axon, a central channel of normally oriented neurofilaments (arrow) is surrounded by a bulbous accumulation of vesicles containing electron-lucent and electron-dense material. ×2,210. B. This distended axon is packed with smooth membranous profiles arrayed in swirls and laminar stacks. A myelin sheath can sometimes be demonstrated around smaller dystrophic axons of this type. ×8,730. C. A higher magnification of the area in the lower right of Figure 4B shows detail of membranous elements. ×21,400.
resolve the genetic basis of the disease. The most significant neuropathological feature, the presence of massive numbers of terminal spheroids filled with particulate organelles, is shared by genetic, neurological diseases in human beings, e.g., NAD (14), in cats (37), and in Suffolk sheep (38). The genetic pattern of the disease in cats is consistent with an autosomal recessive trait, as appears to be the case in infantile NAD and HS (11, 14). The genetics of the disease in sheep is not defined.

Clinical and Laboratory Studies

The clinical manifestations of CNAD are chiefly those of a slowly progressive sensory ataxia beginning in the first year of life. The natural course of the disease is unknown, since it was interrupted by euthanasia at four years, and Rottweiler dogs may live eight to ten years. Dogs with CNAD have decreased proprioception, poor coordination, ataxia, and nystagmus. A diminished response to visual stimuli or touch could not be evaluated.

Infantile NAD begins between six months and three years of life with weakness, hypotonia, and impairment of sensory function, vision in particular. Patients usually die by ten to 12 years of age and are unresponsive to external stimuli, blind, and paralyzed (4, 39). HS has a later onset and a more indolent course characterized by ataxia and extrapyramidal signs with dementia (1, 11). Seitelberger et al. have suggested that infantile NAD and HS may represent a nosological spectrum (40), while others feel that they represent separate entities (4).

Dogs with CNAD had not developed seizures by four years. These have been reported in infantile NAD (4, 6) and HS (11), but may not be a major clinical feature (39). No other biochemical or skeletal abnormalities (other than hip dysplasia) were noted in the affected dogs. One adult dog had an acquired patchy depigmentation of the skin. Huttenlocher and Gilles (7) commented on the pale, translucent skin of their patients, a feature not emphasized by others in describing NAD.

Pathological Features

In both human NAD and the canine disease, cerebellar atrophy and involvement of visual pathways were also recognized. In affected patients, the cerebellums are grossly atrophic and optic nerves quite small; similar changes were observed in affected dogs.

The nature of the pathological changes in CNAD is similar to that described in human NAD (2, 4). The distal portions of specific axonal populations show accumulations of smooth membranes and tubulovesicular profiles. These accumulations produce swelling and eventual degeneration of distal axons and synaptic terminals. By both light and electron microscopy, they are virtually identical to those described in infantile NAD (8, 12, 41). Although peripheral nerve biopsy in one well-documented human patient revealed no changes (7), dystrophic axons were reported in the neuromuscular junction, skin, and conjunctiva of patients with NAD (39). Axonial swellings were not noted in autonomic ganglia, distal peripheral nerves, or the neuromuscular junctions which were examined in CNAD, but more extensive sampling is in progress.

Axonal swellings and the distribution of pathological changes in CNAD were primarily localized to afferent fibers entering sensory nuclei in the spinal cord, brainstem, and diencephalon. In infantile NAD, the presence of spheroids is more widespread with involvement of the cerebral cortex, basal ganglia, and thalamus, as
well as the regions involved in CNAD. Spheroids are accompanied by pigmentary
deposition in HS (11, 14), which is most conspicuous in the basal ganglia, substantia
nigra, and brainstem. No pigmentary changes in CNS tissue were noted in CNAD.

Despite their frequent occurrence (14), the underlying pathophysiology of dystrophic axons is not well understood. This type of pathology occurs in a variety of
settings including aging, exposure to toxins, metabolic diseases, deficiency states,
and genetic disorders. Dystrophic axons and spheroids are commonly seen as a
concomitant of aging in the gracile and cuneate nucleus, zona reticulata, substantia
nigra, and internal pallidum. Statistically, axonal dystrophy in the gracile nucleus
and pars reticulata increases in frequency and severity with age in humans (13) and
dogs (16, 17, 20, 21).

Several chemical toxins, such as tri-o-cresyl phosphate (25–29), ZPT (30, 31),
BPAU (32–35), and diethylidithiocarbamate in sheep (36) can produce membranous
spheroids in distal axons.

A variety of metabolic disturbances, most notably in situations in which there has
been intestinal malabsorption such as in mucoviscidosis, congenital biliary atresia,
and celiac disease (18, 19), are also characterized by the development of dystrophic
axons. Because of the association of dystrophic axons with intestinal malabsorption
syndromes affecting the absorption of fat-soluble vitamins, a role for vitamin E in
these disorders has been hypothesized. Vitamin E-deficient rats develop dystrophic
axonal pathology (22, 42), and vitamin E-deficient dogs also develop somewhat
analogous pathology (23). However, there are species variations in the distribution
and severity of lesions. For example, vitamin E-deficient rhesus monkeys develop
mainly central nervous system lesions (posterior columns) and less severe lesions in
the peripheral nervous system (PNS); no axonal spheroids were seen in the PNS
(24). Serum vitamin E, triglycerides, and cholesterol levels in dogs with CNAD were
normal.

The pathogenesis of the inherited distal membranous axonopathies which occur in
NAD and now CNAD is unknown, although accumulations of smooth membranes
and vesicles, normal constituents of synaptic regions, could implicate a derangement
in the presynaptic portions of the neuron (8) or abnormalities in axonal transport.
The latter hypothesis is suggested by the similarities between dystrophic axons and
reactive axonal swellings following axotomy (12, 43–45). During regeneration, reactive
axonal swellings accumulate densely packed membranous organelles and rapidly
transported proteins. Smooth membranes and vesicles are synthesized in the
neuronal perikaryon and are transported in the axon where they move with the fast
axonal transport system (44, 45). After reaching their destination, some of these
elements turn around and return to the cell bodies by means of retrograde axonal
transport. Thus, accumulations of smooth membranes and vesicles in distal axons
could be explained by: excessive synthesis, impaired utilization by the axon, de-
creased catabolism, and impairment of the “turn-around” mechanism or retrograde
transport. The latter hypothesis has been tested in rats intoxicated with ZPT, and it
appears that there is an impairment of the “turn around” of labeled proteins in distal
axons (31). In BPAU intoxication, studies of axonal transport show a variety of
alterations, the earliest of which is an abnormality of the “turn-around” mechanism
that precedes structural changes (46). Other hypotheses have yet to be explored.
Similar studies of axonal transport, combined with morphological observations,
would be of interest in CNAD, particularly in the preclinical stage. Such studies

might demonstrate perturbations of axonal transport and might define the relationship between such abnormalities and the evolution of morphological change in distal axons.

In summary, CNAD reproduces many of the pathological features of human NAD. The genetic basis of the disease, its slowly progressive course, and its similarities to human inherited NAD make it an excellent model in which to investigate the underlying mechanisms of inherited axonopathies.

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