Congenital Hypomyelinating Polyneuropathy in Two Golden Retriever Littermates

K. G. Braund, J. R. Mehta, M. Toivio-Kinnucan, K. A. Amling, L. G. Shell, and M. E. Matz

The Neuromuscular Laboratory of the Scott-Ritchey Research Program, College of Veterinary Medicine, Auburn University, Auburn, AL; and VA-MD Regional College of Veterinary Medicine, Blacksburg, VA

Abstract. Serial peripheral nerve biopsies from two golden retriever littermates with chronic neurologic disease were taken for morphologic and morphometric evaluation. Teased nerve preparations were difficult to interpret due to the lightness of myelin staining. Light and electron microscopic findings were characterized by the following: reduced number of myelinated axons, presence of myelinated sheaths inappropriately thin for the caliber of the fiber, poor myelin compaction, increased numbers of Schwann cell nuclei, increased concentration of neurofilaments in myelinated axons, many Schwann cells with voluminous cytoplasm, and increased perineurial collagen. Onion bulb formation was not seen. In contrast to control data, a poor correlation was seen between numbers of myelin lamellae (ML) and axonal circumference (AC). The frequency distribution of ML ranged from 5 to 55 lamellae in affected animals (mean, 28 lamellae) compared to 20 to 140 lamellae in controls (mean, 66 lamellae). The MWAC ratio was significantly reduced \((P < 0.001)\) in nerves of affected dogs. Morphometric results indicated that fibers of all calibers were hypomyelinated.

Abnormal myelination of the nervous system has been reported in a variety of animal species, including a number of dog breeds, such as springer spaniels, lurcher hounds, Weimaraners, Samoyeds, and a Dalmatian. Heredity has been implicated as the cause of canine hypomyelinating conditions. In all of these breeds, hypomyelination involved the central nervous system, and peripheral nerves were normal.

In the present report, severe hypomyelination of the peripheral nervous system is described for the first time in two golden retriever littermates.

Materials and Methods

Two golden retriever littermates, one male and one female, were studied from 7 weeks to 5 months of age. Both dogs were initially presented for hind limb ataxia at 7 weeks of age. One other littermate from the litter of seven was reported to be slightly affected but was not examined. Both dogs had a crouched stance, mild pelvic limb atrophy, and weakness. Circumduction was evident in the pelvic limbs when walking, and a "bunny hop" gait was present when running. Segmental reflexes were depressed or absent in pelvic limbs of both dogs and in thoracic limbs of the male. Motor nerve conduction velocities were markedly reduced in sciatic-tibial and ulnar nerves. Needle electromyographic studies revealed rare denervation potentials in a few muscle groups (M.E. Matz et al., unpublished observations).

Under general anesthesia, 2-cm fascicular segments of common peroneal nerve at the level of the stifle were removed, stretched on wooden tongue depressors with pins, and fixed in a mixture of 4% formalin/1% glutaraldehyde. After fixation for 12 hours, nerve samples were divided into halves and washed in Millonig phosphate buffer (pH 7.3) at 4 C overnight. One half was post-fixed in 1% osmium tetroxide for 6 to 8 hours. These samples were then placed in 66% glycerin for 24 hours and stored in 100% glycerin for single teased-fiber preparations as previously described. The other half was post-fixed in 1% osmium tetroxide for 1 hour, washed in phosphate buffer, transferred through graded ethanol solutions, and processed for embedding in Spurr plastic medium. Semi-thin sections (1-2 \( \mu \)m) were cut transversely and stained with paraphenylenediamine. Silver to gray ultrathin sections of nerves from affected and control animals were cut, stained with uranyl acetate and lead citrate, and examined with an electron microscope. Nerve samples were obtained from the male at 7 weeks of age, from the female at 3 months of age, and from both dogs at 5 months of age. Comparable nerve samples, previously obtained from normal, age-matched dogs, and similarly processed, were used as controls. At the same time as the nerve biopsies, muscle samples from standardized sites of the biceps femoris, gastrocnemius, and long head of triceps brachii were obtained without the use of clamps. Each sample was frozen in isopentane, precooled in liquid nitrogen, and stored in airtight plastic bottles at \(-80\) C. Serial sections were cut at 8 \( \mu \)m and stained for routine morphology and histochemistry.

For quantitative studies, transverse sections of nerve were projected onto a monitor using a computerized video-display image analysis system (Optomax semi-automatic image analysis system, Optomax Inc., Hollis, NH). The mean external diameters of the axons that were myelinated were determined from a random field fiber count of approximately 1,500 for
each nerve sample. Random counts were made from four quadrants of each section to avoid the possibility of inadvertently over- or under-sampling large or small fibers. All measurements were made under oil objective at a final magnification of $2,940 \times$. Sections were acceptable for measurement only when the majority of fibers were circular in shape. The diameter of the lesser axis was used on oval fibers. Triangular-shaped fibers were measured across their base. Crenated fibers were not measured. Electron micrographs prepared from nerves collected at 5 months of age were printed at a final magnification of $9,500 \times$ and viewed under a dissecting microscope at about $50 \times$ magnification in order to count the myelin lamellae (ML). The axon circumference (AC) of myelinated fibers was obtained by placing the micrographs on the surface of the digitizer and tracing the periphery of the axon with a magnetic pen. Approximately 250 measurements were made from respective affected and control nerves. The results were recorded in micrometers. For all observations, the numbers of ML and corresponding AC were recorded, and scattergrams with their linear regression lines were plotted. Histograms of absolute axon sizes and ML numbers also were generated. Data were statistically evaluated using the unpaired Student’s t-test.

Results

Morphology

In cross-sectional preparations of control nerves, nerve fascicles were surrounded by a multilamellar perineurium, and fascicles were bound together by epineurial sheaths of variable thickness. Perineurial septa were sometimes present in larger fascicles. Most nerve fascicles contained an admixture of small and larger diameter fibers (Fig. 1). In nerves of the golden retrievers, fascicular organization was less apparent, the number of myelinated axons was markedly less than in controls, and most of the myelin sheaths appeared to be inappropriately thin for the caliber of the fiber (Fig. 2). Axonal density (myelinated plus hypomyelinated) of affected nerves appeared to be less than in control nerves. Compared to controls, many myelinated fibers had eccentric caps of variable proportions. These caps were identified ultrastructurally as voluminous Schwann cell cytoplasm.

In teased nerve studies at the different ages, individual fibers were extremely difficult to prepare because of the lightness of myelin staining. Accordingly, quantitative data pertaining to the incidence of abnormalities were not compiled. However, certain changes were variably seen, including apparent demyelination, questionable presence of intercalated internodes suggesting remyelination, numerous paranodal-like swellings, and, rarely, a degenerating fiber. Ultrastructural examination of nerves revealed a reduced number of myelin lamellae (ML), compared to controls, in most fibers irrespective of fiber caliber (Fig. 3). The number of Schwann cell nuclei seemed increased, compared to control nerves, and “resting” Schwann cells without axons were often seen singly or in pairs. Schwann cell cytoplasm appeared abundant in many cells, often with increased concentration of endoplasmic reticulum, Golgi apparatus, and mitochondria (Fig. 4). Occasional fibers contained a series of incisural openings through the thickness of the myelin sheath. Some fibers showed loose myelin compaction (Fig. 5). There was no evidence of onion bulb formation. Macrophages and denervated Schwann cells were rarely seen. Perineurial collagen was increased. Unmyelinated fibers appeared normal, and the usual axoplasmic constituents, such as mitochondria, microtubules, and neurofilaments, were, in general, well preserved; however, the number of neurofilaments appeared increased (Fig. 3).

Skeletal muscle changes were minimal, characterized by moderate fiber size variation associated with scattered, angular atrophic fibers and multifocal fiber hypertrophy. There was no evidence of regeneration, fiber type grouping, inflammation, or fibrosis. Internalized nuclei, fiber splitting, and focal necrosis were seen infrequently.

Morphometry

Mean diameters were markedly less in affected animals, at each age period (Table 1). The ML/axonal circumference (AC) relationships for approximately 250 fibers, from control and from affected nerves (5 months), were determined and expressed as scattergrams (Fig. 6) and frequency distribution histograms (Figs. 7, 8). There was a good correlation between numbers of ML and AC in controls (correlation coefficient $r = 0.841$). In contrast, a poor correlation was noted in nerves of affected golden retrievers (correlation coefficient $r = 0.149$). The frequency histogram of ML in control nerves ranged from 20 to 140 lamellae, with a mean of $66.11 \pm 2.02$ lamellae and a bimodal distribution (Fig. 7). The frequency histogram of ML in affected nerves ranged from 5 to 55 lamellae, with a mean of $27.53 \pm 0.58$, and a unimodal distribution (Fig. 8). The mean number of ML was significantly less ($P < 0.0001$) in affected nerves compared to controls. The frequency histograms of AC in affected and control nerves (5 months) were very similar, both in numbers and distribution of different caliber fibers (Figs. 9, 10). There was no significant difference in the mean AC of affected and control nerves. To determine if fibers of all axon calibers were equally affected, the ML/AC ratios (mean $\pm$ SE) in affected and control nerves (5 months) were plotted in relation to their absolute AC (Fig. 11). Results indicated that fibers of all calibers, in affected retrievers, were hypomyelinated. The mean ML/AC ratio in affected nerves was significantly less ($P < 0.0001$) than that in controls.
Fig. 1. Control nerve (a) 5 weeks; (b) 5 months. Fascicles containing an admixture of normally myelinated small- and large-diameter fibers. Paraphenylenediamine.

Fig. 2. Affected nerve (a) 7 weeks; (b) 5 months. Most myelin sheaths appear to be inappropriately thin for the caliber of the fiber. Many myelinated fibers have eccentric caps of voluminous cytoplasm (arrows). Paraphenylenediamine.

Discussion

While hypomyelination of the nervous system, specifically the brain and spinal cord, has been described in several breeds of dogs, hypomyelination of peripheral nerves has not been previously reported in domestic animals. Hypomyelination of peripheral and central nervous systems occurs in several mouse mutants, such as shiverer mice, quaking mice, and twitcher and sprawling mice. Hypomyelination of only the peripheral nervous system occurs sporadically in infants and in trembler mice. Another disorder, confined to the peripheral nerves and characterized by the presence of axonal segments totally devoid of Schwann cells, occurs in mutant dystrophic mice.

Clinical signs in our golden retrievers (i.e., ataxia, paresis, muscle atrophy, and hypo-/areflexia) are compatible with a neuropathic syndrome. There was no clinical evidence of central nervous system involvement (M.E. Matz et al., unpublished observations). A diagnosis of peripheral nerve hypomyelination in our dogs was based on the following pathologic findings: paucity of myelinated fibers, fibers with inappropriately thin myelin sheaths relative to the caliber of their enclosed axons, occasional fibers with poorly compacted myelin, Schwann cells with larger than normal cytoplasmic volume, and increased number of Schwann cell nuclei. Similar changes have been reported in children with various forms of congenital hypomyelination and in trembler and trembler-J mutant mice.

The apparent demyelination seen in teased nerve fiber studies was not evident in semi-thin or thin sec-
Fig. 3. Peripheral nerve (a) control (5 months); (b) affected (5 months) with several thinly myelinated fibers (open arrows) and increased perineurial collagen. A “resting” Schwann cell nucleus (block arrow). Increased number of axonal neurofilaments compared to controls. Uranyl acetate and lead citrate. Bar = 2 μm.

Fig. 4. Affected nerve (5 months) with voluminous cytoplasm, prominent mitochondria, and a series of incisural openings through the thickness of the myelin sheath (arrow). Axonal contour is irregular. Uranyl acetate and lead citrate. Bar = 1 μm.

Fig. 5. Affected nerve (5 months) with poor myelin compaction. Uranyl acetate and lead citrate. Bar = 0.5 μm.
Fig. 6. Scattergrams of control and affected nerves (5 months). Myelin lamellae counts plotted against axonal circumference. Linear regression lines for each population are indicated. ○ AC-control, ML-control; • AC-affected, ML-affected.

Fig. 7. Control nerve (5 months). Frequency histogram of myelin lamellae numbers.

Fig. 8. Affected nerve (5 months). Frequency histogram of myelin lamellae numbers.

Table 1. Mean fiber diameters (MFD) of common peroneal nerves from affected golden retriever dogs and controls.

<table>
<thead>
<tr>
<th>Age</th>
<th>Animal</th>
<th>Number</th>
<th>MFD (μm)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 wk</td>
<td>Affected</td>
<td>×1</td>
<td>1.938</td>
<td>0.52</td>
</tr>
<tr>
<td>5 wk</td>
<td>Control</td>
<td>×1</td>
<td>2.872</td>
<td>1.14</td>
</tr>
<tr>
<td>3 mo</td>
<td>Affected</td>
<td>×1</td>
<td>2.711</td>
<td>0.71</td>
</tr>
<tr>
<td>3 mo</td>
<td>Control</td>
<td>×1</td>
<td>4.271</td>
<td>2.21</td>
</tr>
<tr>
<td>5 mo</td>
<td>Affected</td>
<td>×2</td>
<td>3.926</td>
<td>1.37</td>
</tr>
<tr>
<td>5 mo</td>
<td>Control</td>
<td>×2</td>
<td>5.171</td>
<td>2.19</td>
</tr>
</tbody>
</table>

in order to confirm or rule out the teased nerve findings. The degree of difficulty of teased nerve fiber preparations from these affected dogs has not been experienced previously in our laboratory.

Nerves from our dogs manifested a very severe deficiency of peripheral myelination at the three different ages studied. Only a few dozen myelinated fibers were present in some nerve fascicles that normally would have contained hundreds of fibers. It is now evident that intricate Schwann cell-axon interactions occur in normal nerves. Schwann cells have important effects on axons, and axons, in turn, influence normal Schwann cell proliferation, differentiation, and myelin sheath thickness/length. Defects of myelination may result from abnormal Schwann cell function, as reported in trembler and quaking mice, or possibly from abnormal axon-Schwann cell interactions. Many morphologic features seen in our affected dogs are similar to those described in various hypomyelinating mouse mutant models, especially the trembler and trembler-J mutants. The increase in neurofilament concentration of myelinated axons in affected dogs may represent an axonal alteration that is possibly related to the lesions detected. Transplantation experiments in trembler mice have established that the hypomyelination results from a Schwann cell inability to produce or maintain normal myelin. Myelin formation is absent...
or diminished in these mutants, suggesting an impairment of radial and longitudinal extensions of the Schwann cell plasma membranes. It is also feasible that the axons may fail to express a signal for myelin formation, as has been suggested in certain congenital hypomyelinating/amyelinating neuropathies of children. Recent biochemical studies of lipid metabolism and lipid accumulation patterns in trembler nerves during active myelogenesis indicate that the trembler mutation induces an abnormal synthesis/metabolism of peripheral myelin rather than an abnormal degradation/catabolism. In other words, the primary defect is one of dysmyelination rather than demyelination. Use of transplantation models similar to those used in mutant mice and in studies of Tibetan mastiff dogs with hypertrophic neuropathy may help to identify the defect in our dogs. The significant reduction in the number of myelinated lamellae (ML) and ML/axonal circumference (AC) ratios noted in nerves of our dogs has also been reported in trembler and in shiverer mutant mice. No difference in AC was found in affected and control dog nerves. In trembler mice, however, axonal populations are similar to those of controls, but their size is reduced, providing additional evidence for a local effect of Schwann cells on the size of axons. This observation suggests that additional factors may underlie or contribute to the hypomyelination in affected golden retrievers. Unmyelinated fibers appeared to be unaffected in our dogs, similar to findings in trembler and quaking mice.

This canine disease may prove to be a useful model for studying the mechanisms underlying defects of myelination in peripheral nerve. Additional aging studies, biochemical investigations of myelin composition, and breeding trials aimed at clarifying the hereditary/congenital nature of this canine hypomyelinating disorder are planned.

Acknowledgements

This study was supported in part by the Scott-Ritchey Research Fund and the Virginia Veterinary Medical Association Pet Memorial Fund.

References

8. Bunge RP, Bunge MB, Okada E, Cornbrooks CJ: Abnormalities expressed in cultures prepared from periph-

Fig. 9. Control nerve (5 months). Frequency histogram of axon circumference.

Fig. 10. Affected nerve (5 months). Frequency histogram of axon circumference.

Fig. 11. Myelin lamellae/axon circumference ratio results (mean ± SE) from control and affected nerves (5 months) plotted against axon circumference.


Request reprints from Dr. Kyle G. Braund, Neuromuscular Laboratory of the Scott-Ritchey Research Program, College of Veterinary Medicine, Auburn University, Auburn, AL 36849-3501 (USA).