The Dorsal Root Ganglia in Adrenomyeloneuropathy: Neuronal Atrophy and Abnormal Mitochondria

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Abstract. Adrenomyeloneuropathy (AMN), a disease of spinal cord, brain, adrenal, and testis, mostly affects men with spastic paraparesis or ataxia beginning in their second or third decade. The spinal cord displays bilateral, usually symmetrical, long tract degeneration particularly of the gracile tract in a “dying-back” pattern. The available data strongly indicate that the fundamental lesion in AMN is an axonopathy or neuronopathy. We compared lumbar dorsal root ganglia (DRG) from 3 AMN patients to 6 age-matched controls histologically, morphometrically, immunohistochemically, and ultrastructurally. There was no apparent neuronal loss, necrosis or apoptosis, nor obvious atrophy; nodules of Nageotte were sparse in both groups. The morphometric studies, however, did reveal neuronal atrophy with a decrease in the number of large neurons and a corresponding increase in neurons less than 2,000 μm²; especially in the 1,500–1,999 μm² range. No consistent immunohistochemical differences were observed, and no specific cell type appeared to be lost. Many mitochondria in the AMN neurons demonstrated lipidic inclusions; this raises the possibility that, in addition to the well-known peroxisomal defect, impaired mitochondrial function may lead to a failure of ATP-dependent axoplasmic transport in AMN spinal tracts with consequent “dying-back” axonal degeneration. The observation that the DRG parent neurons of the degenerate gracile tracts in AMN undergo atrophy and do not display appreciable evidence of cell death, even at autopsy, provides a wide window of opportunity for the development of therapeutic strategies to combat or prevent this myeloneuropathy.

Key Words: Dorsal root ganglia; Fatty acids; Mitochondrial inclusions; Myelopathy; Neuronal atrophy; Neurotrophins.

INTRODUCTION

Adrenomyeloneuropathy (AMN) is a disease of spinal cord, brain, adrenal, and testis that mostly affects men with symptoms of spastic paraparesis or ataxia beginning in their second or third decade (1, 2). The spinal cord bears the brunt of the neurological disease and is the most consistently affected central nervous system (CNS) structure affected. The clinical and neuropathologic severity of the myelopathy does not appear to correlate with the duration or the severity of endocrine dysfunction or with the presence or absence of cerebral demyelination. Microglia are the dominant responding cells in this noninflammatory myelopathy. The spinal cord lesion consists of bilateral, usually symmetrical, long tract degeneration in which axonal loss is equal to or greater than myelin loss and the severest degeneration is seen in the gracile and corticospinal tracts. The distribution of the gracile and corticospinal tract degeneration conforms to a “dying-back” pattern in that the greatest loss of myelinated fibers is found in the cervical gracile and the lumbar corticospinal tracts. The available data strongly indicate that the fundamental lesion in AMN is an axonopathy or neuronopathy, not a myelinopathy, but the cause of this primary axonal/neuronal degeneration has not been determined.

In order to better understand the fundamental lesion in AMN, we chose to study the cells of origin of 1 of these degenerate tracts, specifically the lumbar dorsal root ganglia that give rise to the gracile tracts. We compared lumbar dorsal root ganglia (DRG) from 3 AMN patients to 6 age-matched controls histologically, morphometrically, immunohistochemically, and ultrastructurally. Preliminary results from some of these efforts have been reported in abstract form (3).

MATERIALS AND METHODS

Lumbar dorsal root ganglia were obtained from 3 AMN patients, 2 of whom have been previously reported by us. Patient #1 was a 22-yr-old male who had endocrinologic problems since adolescence and then developed leg weakness followed by cramps, slowly progressive spastic paraparesis with sensory deficits in both legs, bowel and bladder dysfunction, and death during an Addisonian crisis at the age of 24 (pure AMN). This patient has been previously published by Budka et al (4). AMN patient #2 corresponds to patient LD in our previous publication (2); he demonstrated mild ataxia and a convulsive disorder at the age of 19, followed by spastic paraparesis and adrenal insufficiency at the age of 35 with death at 36 yr of age. At the time of autopsy he demonstrated evidence of confluent cerebral inflammatory demyelinating lesions typical of adreno-leukodystrophy (ALD) (cerebral AMN or AMN/ALD). AMN patient #3 corresponds to patient ML (2); he displayed both adrenal insufficiency and mild ankle clonus at the age of 31, developed spastic paraparesis and ataxia, and died at 51 yr of age. He did not demonstrate any clinical or pathologic evidence of cerebral lesions (pure AMN). Control lumbar DRG were obtained from a 36-yr-old male who died of...
a cardiopulmonary arrest, a 23-yr-old male who died of disseminated Varicella-Zoster infection, a 49-yr-old male who died of an arrhythmia secondary to a myocardial infarct, and a 51-yr-old male who died of acute cardiopulmonary failure due to ischemic heart disease.

**Histologic Staining**

Routine staining with hematoxylin and eosin, Luxol fast-blue and periodic acid-Schiff, Bodian, Gomori trichrome, and cresyl violet was performed. Sections were also cut for morphometric analysis.

**Image Analysis**

The microcomputing imaging device (MCID), Imaging Research, Inc., St. Catherines, Ontario, was used for morphometric analysis. The system utilized an Olympus BH2 microscope with a digital camera to take digital images. An objective magnification of 10× was used, which was calibrated with a stage micrometer. Neurons displaying a clearly identifiable nucleus and nucleolus and cytoplasm immunoreactive with the SMI 31 antibody (phosphorylated neurofilaments) were measured. The SMI 31 antibody appears to identify soluble, phosphorylated forms of the high molecular weight neurofilaments in DRG perikarya (5). More importantly, the immunostain provided sufficient contrast to consistently identify ganglion cells in the MCID; SMI 31 immunoreactive neurons comprised about 94 percent of those present in both groups. The area of each neuron was measured by manually outlining the cell membrane. Each neuron in the section was counted up to a usual maximum of 100 neurons per ganglion. Some sections did not contain that number of neurons with clear nucleoli, so some samples had fewer than 100 measurements (range: AMN, 56–100; controls, 68–132).

Following this analysis, photomicrographs were taken of both groups with the capsule being 1 ordinate and the other ordinate determined by the edge of the optical field at an objective magnification of 4×. These photomicrographs were enlarged into 8 × 10 color photographs, and hand counts of the total number of neurons in the photographs were performed.

**Immunohistochemical Staining**

Immunohistochemical staining was performed with commercially available antibodies at appropriate dilutions in a streptavidin-biotin system using diaminobenzidine (DAB) or amino ethyl carbazole (AEC) as chromogens. Positive and negative controls accompanied all of these. None of these proved to be informative because the AMN-DRGs did not label differently from the controls in respect to intensity of staining or in the distribution or number of immunoreactive cells. Hence, only a few results accompanied all of these. None of these proved to be informative because the AMN-DRGs did not label differently from the controls in respect to intensity of staining or in the distribution or number of immunoreactive cells. Hence, only a few results will be reported and discussed. The following antibodies and immunoreagents were used: 25F9 (BMA Biomedical, King of Prussia, PA), AEC (Scytek Laboratories, Logan, UT), Androgen Receptor (Affinity BioReagents, Neshanic Station, NJ), APP-A4 (Boehringer Mannheim, Indianapolis, IN), β-2 microglobulin (Dako, Carpinteria, CA), bcl-2 (Dako), Biotinylated Secondary Abs (Vector Laboratories, Burlingame, CA), Calbindin-D 28K (Sigma Chemical Company, St. Louis, MO), Calcitonin (Dako), Catalase (The Binding Site, San Diego, CA), CD 45-RO (Dako), CD 68 (Dako), c-fos and c-jun (Santa Cruz Biotechnology Inc., Santa Cruz, CA), Choline acetyltransferase (Chemicon International Inc., Temecula, CA), CLA (Dako), cytochrome D1 (Santa Cruz Biotechnology Inc.), Cytochrome oxidase (Molecular Probes Inc., Eugene, OR), FGF receptor (Zymed Laboratories Inc, So. San Francisco, CA), Ganglioside-Cholera toxin (Matreya Inc., Pleasant Gap, PA), GFAP (Dako, Carpinteria, CA), HAM 56 (En Diagnostics, Farmingdale, NY), HNK-1 (Becton Dickinson, San Jose, CA), HSP 27 and 70 (StressGen Biotechnologies Corporation, Victoria, British Columbia, Canada), IL-1α (Genzyme, Cambridge, MA), Ki-67 (Dako), L3-3 (Bio Genex, San Ramon, CA), Lysozyme (Dako), MAP kinase (Zymed Laboratories Inc.), MEK-1 (Santa Cruz Biotechnology Inc.), Neurofilament (Dako), Nonspecific esterase (Biogenesis Ltd., Kingston, NH), Neuron Specific Enolase (Dako), p53 (Santa Cruz Biotechnology Inc.), Parvalbumin (Sigma Chemical Company), PCNA (Santa Cruz Biotechnology Inc.), Proteolipid protein (Immunodiagnostics Inc., Bedford, MA), S-100 (Research Development Corporation, Toronto, Canada), SMI 31 and 32 (Sternberger Monoclonals Inc., Lutherville, MD), Somatostatin (Dako), Streptavidin Peroxidase Conjugate (Jackson ImmunoResearch Laboratories, West Grove, PA), Substance P (Incstar, Stillwater, MN), Synaptophysin (Dako), TNF-a (Genzyme), Trk A, B, C (Santa Cruz Biotechnology Inc.), and Ubiquitin (Chemicon International Inc.).

**Apoptotic Assays**

Three separate attempts to detect apoptosis were made: Apop Tag Peroxidase Assay Kit (Oncor) as performed in the laboratory of Harris Gelbard, MD, PhD (6); in situ end labeling as performed in the laboratory of Dennis Steindler, PhD (7); and in situ end labeling (Boehringer Mannheim) performed in our laboratory according to the vendor’s directions. Appropriate positive controls were included (e.g. lymphoma or fetal brain).

**Ultrastructural Analysis**

A lumbar DRG from AMN patient #2 was initially fixed in 4% buffered glutaraldehyde, postfixed in 2% osmium tetroxide, and processed routinely for electron microscopy. Two additional age-matched controls were processed similarly. One control was a 58-yr-old male with a history of Crohn’s disease, who died of rhinocerebral mucormycosis. The other was a 44-yr-old male who had a back injury about 7 yr before death necessitating 3 lumbar laminectomies and spinal fusion. He also was noted to have extremely highly serum triglycerides and abnormal liver functions before a metastatic cholangiocarcinoma was found at T11, T4, and T3. He received radiation therapy to the spinal column about 1 wk before death. He died of an apparent myocardial infarct. After the ultrastructural examination of the DRG, the DRG epoxy blocks from the AMN patient and 1 control were recut; unstained, thin sections mounted on nickel grids were subjected to electron probe microanalysis in a Phillips CM12-EDAX 2 and subsequently in a JEOL 2000 FX analytical transmission electron microscopes by Mr. Jack Czerniawski of the Xerox Corporation. Following this examination, the other 2 AMN-DRG patients and 1 of the age-matched controls were subjected to ultrastructural examination by using the “pop-off” technique (8).
Supplementary Studies

Finally, all the AMN- and control DRG were stained with alizarin red to detect calcium, Prussian blue reaction to detect iron, and rubeanic acid to detect copper, and were immunostained again with the antibodies to phosphorylated neurofilaments, cytochrome oxidase, parvalbumin, and calbindin.

RESULTS

By routine staining the AMN-DRG were microscopically indistinguishable from the controls. In particular, there was no apparent neuronal loss, necrosis, apoptosis or obvious atrophy, and nodules of Nageotte reflecting cell loss were sparse in both groups. No difference in nuclear or nucleolar size could be appreciated; the amount of cytoplasmic pigment (lipofuscin) was comparable in both groups. Occasionally, a few lymphocytes were noted in the stroma of both groups. The alizarin red stain for calcium, the Prussian blue reaction for iron, and the rubeanic acid stain for copper failed to distinguish the AMN- from control DRG.

As alluded to earlier, the immunohistochemical results were disappointing in that no staining difference could be appreciated between the AMN- and control DRG. On the other hand, the immunohistochemical data suggest that no specific neuronal population (e.g. parvalbumin-containing or trk C-positive ganglion cells) was lost in AMN. There was an equivocal decrease in phosphorylated neurofilament immunoreactivity in the DRG from the AMN patients. It is important to emphasize that there was neither evidence that AMN ganglion cells entered the cell cycle nor that they underwent apoptosis, despite the latter assay being performed in 3 different laboratories.

The morphometric studies, however, revealed a decrease in the number of large neurons in the AMN patients and a corresponding increase in the population of neurons less than 2,000 \( \mu m^2 \), especially in the 1,500–1,999 range (Figs. 1, 2). Figure 1 compares the 2 groups using a number of characteristics of the distributions for the individual subjects. The subjects are numbered on the abscissa (AMN patients, 1–3; controls, 1–4). Repeated samples for the same subject are numbered using the letters A–C with the same subject number in the figure. Repeated samples for the same subject give an indication of variability within the same individual. Each distribution is summarized using a box or rectangle. The upper edge of the box marks the 75th percentile of the distribution; the lower edge is the 25th percentile. Thus, the extent of the box indicates the middle 50% of the distribution, with 25% of the distribution above the box and 25% below the box. The length of the box is a measure of variability of the distribution (the interquartile range). The median of the distribution is indicated by a horizontal segment inside the box. The vertical line segments or whiskers above and below each box indicate the extremes of the distribution. Points above or below the extremes are labeled as outliers. In addition to the individual box plots, a horizontal line at 2,000 \( \mu m^2 \) has been added to help visualize differences among the distributions. The distributions for the 3 AMN patients have been placed on the left-most part of the graph, with the 4 controls on the right; a large vertical dashed line separates the 2 groups. Within these groups, subjects are ordered approximately by the size of the median of the distribution.

AMN patient #1 is a patient with a small median (1647), whose 75th percentile (2081) is slightly above...
TABLE

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Average Neuronal Counts/Area

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2,000 μm². Control patient #4 is a control with the largest median (2279). AMN patient #3 is a patient whose values are intermediate between the 2 other AMN patients and the controls. Distributions for AMN patients clearly have smaller cell sizes than controls. This is seen not only in the median, but in other descriptive parameters of these distributions as well. In order to have a statistical comparison of the 2 groups, a Mann-Whitney test comparing the medians of the distributions in the 2 groups was performed. Repeated samples for the same subject were combined into a single group so that a median could be computed for each subject. An exact, 1-sided p value for this test of significance was p = 0.048.

In view of the absence of signs of neuronal necrosis and obvious neuronal loss in the AMN-DRG and to strengthen our data indicative of neuronal atrophy, we also counted the total number of neuronal perikarya per unit area. In addition to the AMN-DRG having the same or a greater numbers of neuronal perikarya per unit area, neuronal atrophy in the AMN-DRG became apparent when the photographs were placed side by side (Table 1; Fig. 3). As with previous data, multiple observations on some subjects were combined (averaged) to have a single observation for each subject. The exact, 1-sided (lower cell numbers corresponding to larger cells) p value for the Mann-Whitney test comparing the 2 groups was 0.065. The spinal cord sections from our patients (AMN patient #2 and #3) were also compared. Both AMN patients displayed a “dying-back” axonopathy affecting the gracile tracts; the loss of myelinated fibers was greater in AMN patient #2, who also had more atrophic DRG neurons (Fig. 4).

Ultrastructural examination of the prospectively fixed specimen from AMN patient #2 demonstrated an equivocal increase in the amount of lipofuscin when compared to the controls; but, as mentioned above, this was not confirmed at the light microscopic level where sampling is less of a problem. Additionally and most importantly, many mitochondria in the AMN neurons available for study demonstrated large, round, occasionally multiple, sharply delimited electron-dense inclusions (Fig. 5). These have been referred to as lipidic inclusions (9). All but 1 of the controls lacked lipidic inclusions; but they all had the common and typical flocculent densities that are seen within almost all postmortem mitochondria (10). One control did exhibit a few mitochondria with lipidic inclusions; he also demonstrated a mild, bilaterally symmetrical degeneration of the medial portion of the cervical gracile tracts. This was the control who had metastatic cholangiocarcinoma, had been subjected to previous lumbar surgery and underwent therapeutic radiation to his lower back approximately 1 wk before death. The electron probe microanalysis of AMN patient #2 did not reveal iron, calcium, or phosphorous in the lipidic inclusions of the mitochondria, but the copper and magnesium peaks were equivocally elevated. These elevations were considered to be nonspecific and insignificant by Mr. Czerniawski.

DISCUSSION

The present study has further clarified the nature of the myelopathy in AMN. The most recent and extensive neuropathologic investigation of the myelopathy in AMN provided convincing evidence that the lesion is a tract degeneration, particularly involving the gracile and corticospinal tracts, and consistent with either a central-peripheral distal (“dying-back”) axonopathy or a neuronopathy (2). The present data support the notion that in AMN, as in numerous neurotoxic-neurometabolic settings, the largest and longest axons in the spinal cord sustain the greatest damage. They also show that the large neurons of the DRG undergo atrophy, but the DRG do not exhibit appreciable neuronal loss in AMN. A second and unexpected major finding was the ultrastructural demonstration of abnormal mitochondria. The therapeutic implications of these findings will be discussed below.

Our data suggest that the DRG does not exhibit appreciable neuronal loss in AMN. We base this conclusion on the sum total of our results. These include the lack of apoptosis or necrosis, the lack of nodules of Nageotte, the increased neuronal density in AMN DRG, and the decrease in the number of large neurons with a disproportionate increase in the number of intermediate and small neurons. The least compelling data are our morphometric counts of neurons per area. We were not able to use an unbiased stereological method to measure total neuronal number because the tissue that was available to us was in paraffin blocks. The counts that we did perform were the next best available option. These counts were performed by 1 observer in a blinded fashion. Multiple counts per individual were performed with good reproducibility. The values obtained demonstrate that neuronal density was actually increased in AMN compared to controls. This is consistent with the neuronal atrophy that
was present both by observation and by morphometric measurement. We cannot completely rule out the possibility that this increased density could be associated with a decrease in the total number of neurons per DRG if the volume of the DRG were sufficiently reduced. Future studies are necessary to verify whether or not the total number of neurons is preserved by doing an unbiased stereological analysis on appropriate tissue samples.

In our previous studies (2, 11) we drew analogies between the myelopathy of AMN and that of Friedreich’s Ataxia because a “dying-back” pattern of axonal degeneration was seen in the posterior columns, corticospinal...
tracts, and dorsal spinocerebellar tracts of both diseases and the large myelinated fibers seemed to be the most susceptible (12). However, the present examination of the AMN-DRG confirms earlier reports from a few patients that AMN-DRG do not demonstrate neuronal loss (1, 13); this contrasts markedly with the DRG of Friedreich’s Ataxia in which neuronal loss and nodules of Nageotte are prominent (14). A comparable loss of DRG neurons is seen in a few other rare degenerative diseases in which a similar myelopathy dominates, such as Biemond’s Ataxia (15) and Hereditary and Sensory Neuropathy Type II (16). Hence, these other diseases at autopsy appear to be more neuronopathic, at least in respect to their DRG, while AMN is more axonopathic. AMN-DRG neurons do not appear to be lost, even after decades of a progressive clinical myelopathy, which makes therapeutic intervention a realistic possibility.

Our morphometric data indicate that the greatest damage involves the large DRG neurons; this is to be expected, because they are generally considered to give rise to large myelinated proprioceptive afferent fibers that innervate skeletal muscle, tendons, and joints, and are the major occupant of the posterior columns. Impairment in their function leads to sensory ataxia. These neurons are reported to be immunoreactive with antibodies to parvalbumin and carbonic anhydrase (17, 18). The large DRG neurons in rodents display trk C receptors (19, 20), whose preferred neurotrophic ligand is neurotrophin (NT)-3 (20, 21). The parvalbumin- and trk C-antibodies did label large (not all) and medium neurons in our control DRG, but occasionally also labeled small ganglion cells; their immunoreactivity was not consistent enough to allow us to compare morphometrically the parvalbumin or trk C neurons in both groups. However, there did not appear to be any quantitative or qualitative difference with either immunostain between groups by visual inspection. Knockout trk C (22) and NT-3 (18) mutant mice display abnormal limb movements due to the loss of large DRG neurons and large myelinated fibers.

In many ways the myelopathy and the DRG of AMN also resemble those seen in chronic cisplatin intoxication. In humans treated with cisplatin for malignancies, loss of myelinated fibers and gliosis of the posterior columns with loss of large myelinated fibers in sural nerve have been observed (23). The mean volume of DRG soma also was reduced (by 18%), but additionally there were some necrotic neurons and nodules of Nageotte (24). In rats chronically intoxicated with cisplatin the same changes in DRG and peripheral nerve were observed (25–28), as well as the adaxonal abnormalities seen in AMN (2, 27). Three studies suggested that alterations in the nucleoli of DRG neurons might play a role in its neurotoxicity (25–27), but at present the mechanism of cisplatin neurotoxicity is unknown. Some believe it differs from the chemotherapeutic mechanism that depends upon cisplatin binding to DNA (29). Of interest in the context of the “dying-back” axonopathy typical of AMN and the mitochondrial abnormalities described above, “the drug does interfere with axonal transport in vitro or in vivo” (29). Another study implicates an abnormality in slow axoplasmic transport because of an abnormal cytoplasmic distribution of neurofilament protein in large DRG sensory neurons; these effects could be reversed or prevented by treatment with NT-3 (28).

The lipidic inclusions noted within DRG neuronal mitochondria of all 3 AMN patients are worthy of comment. Firstly, it should be noted that these lipidic inclusions have not been mentioned or illustrated in several large ultrastructural studies of normal or aged human DRG (30, 31) or sympathetic ganglia (32–34), which agrees with our normal control DRG. However, they have been observed in a surgically removed lumbar sympathetic ganglion of a 54-yr-old male with chronic lead poisoning, which the authors considered a “degenerative” change (32). The precise chemical composition of lipidic inclusions is unknown, but they are believed to be largely composed of lipid and perhaps some protein (9). Our electron probe microanalysis of these inclusions in AMN is consistent with such a chemical composition. They occasionally can be seen in mitochondria of normal rodent brown fat, adrenal cortex, kidney, or liver (35). In other locations and situations they appear to be pathologic and are believed to be derived from “disintegrating cristae caused by some noxious influence” (9). Some classify them as a secondary abnormality, which are distinct from the paracrystalline arrays and other mitochondrial abnormalities seen in primary diseases of mitochondria. They might indicate “uncoupling of respiration and oxidative phosphorylation, or an indication of defective mitochondrial function” (35). Lipidic inclusions have been observed within abnormal Purkinje cells and several other neuronal populations in Menkes Disease (36, 37), where a genetic defect of copper metabolism leads to decreased activity of the copper-dependent mitochondrial enzyme, cytochrome c oxidase. They also have been identified in neurons of an ataxic canine encephalomyelopathy suspected to be a mitochondrial disease (38). Most relevant to AMN is the presence of lipidic inclusions within DRG mitochondria of focal cytochrome c oxidase deficiency due to a unique point mutation in mitochondrial tRNA{ile} (nt 4269) (39). Even though the CNS of this patient was stated to be histologically unremarkable, the finding of abnormal mitochondria in all 3 AMN patients raises the possibility that abnormal mitochondrial function leads to a failure of ATP-dependent axoplasmic transport in AMN spinal tracts and consequent axonal degeneration.

ALD and AMN have an X-linked, peroxisomal enzyme defect in very long chain fatty acids (VLCFA) synthetase, resulting in a diagnostic accumulation of
VLCFA in blood and a few target organs; however, several key biochemical and molecular questions remain (40, 41). For example, the precise relationship of the ALD/AMN X chromosome encoded gene product, ALDP (an integral peroxisomal membrane protein of the ABC hemi-transporter class), to the deficient synthetase activity is still unknown. Furthermore, the molecular defect in the ALD/AMN gene does not correlate with the type or severity of the clinical phenotype (42–44). This has raised the possibility of a role for modifier autosomal genes (45) or environmental factors in the pathogenesis of ALD and AMN. There is also some evidence that these patients have enhanced microsomal chain elongation activity, which also contributes to the excess of VLCFA (46). Perhaps ALDP has another but still unknown function; and this anabolic system may play a greater pathogenic role than we have previously suspected. To identify mitochondrial abnormalities in AMN further complicates our pathogenetic formulation. However, mitochondrial abnormalities in a peroxisomal disease should come as no surprise. The first peroxisomal disease identified in man, the cerebro-hepato-renal syndrome of Zellweger, also was found to have mitochondrial abnormalities (47). Both peroxisomes and mitochondria carry out the β-oxidation of fatty acids: VLCFA in peroxisomes and shorter forms in mitochondria. In fact, the peroxisome initiates the β-oxidation of VLCFA and mitochondria complete the process (48). Mitochondrial abnormalities also have been reported in ALD: decreased histochemical activity of a mitochondrial enzyme, menadione-dependent alpha-glycerophosphate dehydrogenase, in adrenal cortex, and lamellar-out mitochondrial membrane contacts in the same cells at the ultrastructural level. No intramitochondrial lipidic or paracrystalline inclusions were noted, but a marked pleomorphism of adrenocortical mitochondria was observed in ALD; the lack of sufficient normal age-matched control adrenals did not allow us to conclude that this was a pathologic alteration (49). Some dysfunction of the mitochondrial phase of steroidogenesis in the adrenal cortex, the 11-hydroxylase system, also has been reported in ALD (50).

These data are consistent with our working hypothesis that VLCFA in ALD/AMN become incorporated into lipid and protein components of cell membranes and perturb their microenvironments, leading to cellular dysfunction or membrane instability (11). One of us (JMP) has found VLCFA-gangliosides in axonal membranes to be particularly attractive for explaining the “dying-back” myeloneuropathy of AMN by interfering with normal and necessary trophic interactions (2, 11, 51). Indeed, there is experimental evidence that adult DRG or sympathetic neurons and their axons undergo atrophy and loss if they are deprived of neurotrophic factors, such as nerve growth factor (52, 53).

Therapeutic Considerations

AMN patients do not develop their first myeloneuropathic signs until the second or third decade, and at least 1 set of affected parent neurons appear to be preserved at their deaths years to decades later; these findings provide an incredible opportunity for therapeutic intervention, either preventive or ameliorative. Based on our neuropathologic data and experimental evidence derived from a number of diverse studies, it would seem prudent to pursue a multipronged therapeutic approach. Firstly, the documented adverse effect of VLCFA on membrane structure and function, in vivo and in vitro (42, 49), justifies the reduction of VLCFA from blood and particularly from CNS in these patients. There appears to be some difficulty for erucic acid (Lorenzo’s oil), which dramatically lowers VLCFA levels in blood, to gain access to the CNS through the blood-brain barrier (54). However, some compounds containing erucic acid that rapidly cross the blood-brain barrier have been synthesized recently and are being tested in our laboratory. Secondly, if an axonal membrane-trophic factor dysregulation does exist in AMN, then treatment with neurotrophic factors, particularly NT-3, might be beneficial. Treatment with normal gangliosides is, at least theoretically, a reasonable approach; but the possibility of inducing an injurious immunologic reaction to both exogenous and subsequently to endogenous gangliosides dampens our enthusiasm for this approach. One should recall that N-acetyl-L-cysteine, a molecule that alone or in combination with vitamins C and E seems to protect neurons against trophic factor deprivation, has a safe and long history of therapeutic application (55). Thirdly, in view of the abnormal DRG mitochondria just identified in AMN patients, and the possible adverse effect that this might have on axoplasmic transport in long spinal tracts, a general mitochondrial or antioxidative therapy may be warranted (e.g. L-carnitine, biotin, α-lipoic acid, vitamin C, selenium). Finally, it might be relevant that ACTH 4–9 can prevent the abnormality in axoplasmic transport produced by cisplatin in murine neuroblastoma cell lines (56) and has been used as a neuroprotectant with mixed results in humans (57). Consequently, pharmacologic treatment with ACTH 4–9 might merit some consideration.

In summary, we have demonstrated that the DRG parent neurons of the degenerate gracile tracts in AMN undergo atrophy and do not display appreciable evidence of cell death, even at autopsy. This provides a wide window of opportunity for the development of therapeutic strategies to prevent or combat this myeloneuropathy. The ultrastructural finding of abnormal mitochondria in these cells raises the possibility of a concomitant pathogenic mitochondrial lesion in the spinal tract degeneration of AMN.
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