Hereditary Striatonigral and Cerebello-Olivary Degeneration of the Kerry Blue Terrier. II. Ultrastructural Lesions in the Caudate Nucleus and Cerebellar Cortex

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Abstract. The character and progression of ultrastructural lesions in the caudate nucleus and cerebellar cortex were studied in four Kerry Blue Terriers afflicted with a hereditary neurodegenerative disease. In the caudate nucleus, the initial lesion was mitochondrial hypertrophy in dendrites of intrinsic neurons. Degeneration of these neurons became widespread while axons of passage and terminal boutons were spared. During the final stages, there was severe disruption of the neuropil with loss of both neurons and glia. A narrow zone bordering the lateral ventricles, however, remained unaffected. In the cerebellar cortex, the lesions involved principally Purkinje cells and progressed through a pattern of degeneration comparable to that involving intrinsic neurons of the caudate nucleus. In the later stages, there was astroglial scarring of the molecular layer. In contrast to the caudate nucleus, there was no disruption of the neuropil with loss of structure in the cerebellum. The fact that progression of lesions during the early stages of the disease in both the caudate nucleus and cerebellar cortex was similar suggested a common mechanism for the neurodegeneration.

Key Words: Caudate nucleus; Cerebellar cortex; Dog diseases; Electron microscopy; Hereditary diseases; Neurodegenerative disease.

INTRODUCTION

Striatonigral and cerebello-olivary degeneration of the Kerry Blue Terrier is a neurodegenerative disease with juvenile onset and simple autosomal recessive inheritance. Onset is from nine to 16 weeks (1) or up to 22 weeks (2) of age. Clinical signs are principally cerebellar ataxia with spasticity (3). Central nervous system lesions characteristic of the disease are progressive cerebellar cortical atrophy, evident at the onset of clinical signs, followed by neuronal degeneration in the caudate nucleus and putamen at approximately seven months of age (1-3). The caudate nuclear lesions, which primarily involve the body and head of the nucleus, progress to cavitation by 11 to 12 months of age (2). A narrow subependymal zone in these areas and the tail of the caudate nucleus remain unaffected (2). Involvement of the putamen may be variable as it has been reported in only one dog (2). Neuronal degeneration also occurs in the caudal olivary nucleus (1-3) and substantia nigra (1-4) but damage in these areas is thought to be secondary to the neuronal degeneration in the cerebellar cortex and basal nuclei, respectively (2, 3). Ultrastructural studies have not been reported.

This study was conducted to characterize the morphology and progression of ultrastructural alterations in the caudate nucleus and cerebellar cortex in four dogs at various stages of the disease.

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TABLE 1
Sex, Duration of Clinical Illness, Age at the Time of Autopsy and Type of Fixative used for Perfusion in Dogs Studied

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Sex</th>
<th>Duration of clinical illness</th>
<th>Age at the time of autopsy</th>
<th>Type of fixative used for perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>70352</td>
<td>Female</td>
<td>1 week</td>
<td>6 months</td>
<td>Formal-glutaraldehyde</td>
</tr>
<tr>
<td>67497</td>
<td>Female</td>
<td>2 weeks</td>
<td>5 months</td>
<td>Formal-glutaraldehyde</td>
</tr>
<tr>
<td>62777</td>
<td>Female</td>
<td>2.5 months</td>
<td>7 months</td>
<td>10% buffered formalin</td>
</tr>
<tr>
<td>64015</td>
<td>Female</td>
<td>7.5 months</td>
<td>12 months</td>
<td>Formal-glutaraldehyde</td>
</tr>
<tr>
<td>62305a</td>
<td>Female</td>
<td>Control</td>
<td>6 months</td>
<td>10% buffered formalin</td>
</tr>
<tr>
<td>68502b</td>
<td>Male</td>
<td>Control</td>
<td>Adult</td>
<td>Formal-glutaraldehyde</td>
</tr>
<tr>
<td>R00190b</td>
<td>Male</td>
<td>Control</td>
<td>Adult</td>
<td>Formal-glutaraldehyde</td>
</tr>
</tbody>
</table>

* Mixed breed dog.

b Clinically normal Kerry Blue Terrier.

MATERIALS AND METHODS

Four affected Kerry Blue Terriers from three litters and ranging in age from five to 12 months were included in this study. One clinically normal adult Kerry Blue Terrier and two mixed-breed dogs approximately six months old served as controls. The animal numbers, sex, duration of clinical illness, age at the time of autopsy and fixatives used during the processing of tissues are given in Table 1. A complete description of the gross and light microscopic central nervous system lesions in these four dogs has been reported (2).

All affected Kerry Blue Terriers and control dogs were fixed by perfusion using either formol-glutaraldehyde (5) or 10% neutral buffered formalin via a cannula placed through the left ventricle and into the brachiocephalic trunk as previously described (2). Following perfusion, samples of visceral organs were removed and immersed in fixative. The carcasses, with brains and spinal cords intact, were placed in a refrigerated room at 4°C for 18 to 24 hours after which time the brains and spinal cords were removed and immersed in fixative. For electron microscopy, tissue blocks one cubic millimeter in size were removed from the head of the caudate nucleus and from the vermis, paramedian lobule, and lateral hemispheres of the cerebellar cortex. Blocks were post-fixed in 1% osmium tetroxide and stained ‘en bloc’ with 2% uranyl acetate. Thin sections were stained with uranyl acetate and lead citrate for ultrastructural examination.

RESULTS

Fixation of the brains in all dogs was judged to be adequate. Despite the fact that all dogs were processed in a similar manner, there was some variability in the degree of fixation. Changes such as splitting or separation of myelin sheaths and occasional membranous whorls within cells and their processes as well as in the extracellular space were considered artifactual. The ultrastructural lesions described in this report were not seen in any of the control dogs.

Although chronologically the initial brain lesions involved the cerebellar cortex in all dogs, the progression of lesions in the caudate nucleus followed a more well-defined temporal course. This was due largely to the fact that cerebellar lesions had already begun and, in some cases, were far advanced by the time clinical signs were noted. For this reason, a clearer understanding of the progression of brain lesions can be attained by beginning the results with a description of the lesions in the caudate nucleus.

Caudate Nucleus

The ultrastructural lesions in the caudate nucleus were grouped into three stages, predegenerative, active degeneration, and postdegenerative. The predegenerative stage was studied in dogs 67497 and 70352. During this stage, there were no significant light microscopic lesions and ultrastructurally, only mild alterations were detected. During the degenerative stage (dog 62777) active degeneration of neurons was seen in the central core of the head of the nucleus with both light and electron microscopy. In the postdegenerative stage (dog 64015) active degeneration was no longer evident but the nucleus was severely disrupted due to loss of both neuronal and glial elements with resultant cavitation at both the light microscopic and ultrastructural levels.

During the predegenerative stage, only mild lesions were detected in neurons and dendrites of the caudate nucleus. Multiple lipid inclusions of varying size and electron density occurred in the cytoplasm of neuronal cell bodies and dendrites. In some neurons the inclusions were partially surrounded by a single unit membrane. Dog 67497 also had homogeneous osmiophilic intramitochondrial inclusions in the perikarya of a few large neurons. The inclusions were discretely demarcated and were variable in size, some almost filling the mitochondrial matrix. Other degenerative changes in neurons bearing these inclusions were not detected. The remaining neurons and glia, as well as the vasculature, were normal.

During the degenerative stage of the disease, there was a progressive increase in the severity of the lesions, which was relatively mild at the periphery of the head of the nucleus and progressed to severe destruction in the central core. At the periphery, dendritic processes contained enlarged mitochondria in contrast to the normal mitochondria of surrounding terminal boutons and axons (Figs. 1–3). The enlarged mitochondria had an electron-dense granular matrix and numerous cristae. In addition, many of these dendrites appeared swollen with dispersion of cytoplasmic organelles (Fig. 2) and some contained lipid inclusions (Fig. 1). Contracted, electron-dense degenerating dendritic profiles, identified by their asymmetrical synaptic contacts with normal terminal boutons, were scattered throughout the neuropil (Fig. 2). Astrocytic cell bodies and processes were swollen and there was dispersion of relatively normal cytoplasmic organelles (Fig. 4). Numerous terminal boutons, axons, and dendrites remained unaffected in this area of the nucleus.

Toward the center of the nucleus the number of degenerating dendritic profiles increased. The degenerating dendrites contained enlarged mitochondria, as previously described, flocculent osmiophilic densities, and had a granular cytoplasmic matrix. Myelinated and unmyelinated axons and terminal boutons, many of which made asymmetrical synaptic contacts with degenerating dendrites, were unaffected. Astrocytic cell bodies and processes remained swollen and contained glycogen granules.

At the center of the head of the nucleus, degenerating neuronal cell bodies were occasionally present and severe degenerative changes had altered the morphology of the neuropil. Cell bodies of degenerating neurons had contracted, irregularly shaped nuclei with marginal clumping of the nuclear chromatin (Fig. 5). Cytoplasmic organelles were decreased in number with only a few contracted, electron-dense mitochondria identifiable. The cytoplasm was granular and electron-dense and, in some, there were lipid inclusions. The surrounding neuropil was severely disorganized due to degeneration and loss of dendritic processes as well as swelling of astrocytic cell bodies and processes and there was abundant extracellular space (Figs. 6,
Fig. 4. Periphery of caudate nucleus (dog 62777). A swollen astrocyte (A) partially surrounds an oligodendrocyte. A dendrite contains enlarged mitochondria (arrow). × 10,300.

7). Swollen dendrites containing enlarged mitochondria and contracted, electron-dense degenerating dendrites were scattered throughout the neuropil. Morphologically normal axons and terminal boutons were numerous. Rarely, electron-dense degenerating axons containing contracted mitochondria and degenerating organelles were encountered in this area of the nucleus. Oligodendroglia and the vasculature were unaffected.

In the postdegenerative stage, the entire head of the nucleus was severely degenerated except for a narrow subependymal zone bordering the lateral ventricle. At this stage, all cell types, including neurons and glia, were severely depleted and the neuropil consisted of a structureless array of degenerated organelles, amorphous debris, and membrane-bound aggregates of membranous bodies and flocculent densities (Fig. 8). Astrocytic and neuronal processes criss-crossed the neuropil. Cell bodies with the nuclear and cyttoplasmic characteristics of neurons were also occasionally encountered and were relatively normal even though the surrounding neu-

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Fig. 1. Periphery of caudate nucleus (dog 62777). Dendrites contain lipid inclusions and enlarged mitochondria (arrows) which have a dark granular matrix and numerous cristae. Compare with mitochondria in surrounding axons and dendrites. × 13,700.

Fig. 2. Periphery of caudate nucleus (dog 62777). A swollen dendrite (D) contains an enlarged mitochondrion. A degenerating dendrite (arrow) makes asymmetrical synaptic contacts (arrowheads) with two normal terminal boutons. × 13,700.

Fig. 3. Higher magnification of mitochondrial abnormalities described in Figures 2 and 3 (dog 62777). × 27,400.
Fig. 5. A degenerated neuron containing a cytoplasmic lipid inclusion (L) in the central core of the caudate nucleus (dog 62777). There is margination of nuclear chromatin (open arrows) and loss of cytoplasmic organelles with only a few mitochondria discernible (arrows). × 20,400.

ropil was severely degraded (Fig. 9). Reactive astrocytes and macrophages containing lipid phagosomes and lipofuscin were scattered throughout the area of degeneration. Oligodendroglia were not detected. The few blood vessels that were encountered were relatively normal with an intact basal lamina and continuous endothelial lining. Fragmented astrocytic processes formed a discontinuous lining around the basal lamina. The border between the severely affected part of the nucleus and the normal subependymal zone was irregular but sharply demarcated and there was no prominent intervening zone of astrocytic proliferation or scarring. Neuronal and astrocytic cell bodies and processes were swollen, but cytoplasmic organelles and nuclei were normal. Oligodendroglia were unaffected and the ependymal lining was normal.

Cerebellar Cortex

Lesions in the cerebellar cortex were studied in dogs 67497, 70352, and 62777. The three stages of degeneration described in the caudate nucleus were not applicable to the cerebellum because all dogs included in this study had active degenerative lesions in this area of the brain. The lesions in the cerebellar cortex, however, had a progressive pattern of active degeneration that was quite similar to the progression of lesions in the caudate nucleus. Lesions in the cerebellar cortex began earlier and progressed from the vermis and paramedian lobule to the lateral hemispheres. Dogs with clinical signs for only one to two weeks (70352 and 67497) had advanced degeneration of the vermal cortex with relatively less severe lesions in the lateral hemispheres. After a longer period of clinical illness (dog 62777), the lesions in the vermis and paramedian lobule were far advanced while those in the lateral hemispheres were in both early and later stages of development. To avoid redundancy,
Fig. 6. Severely degenerated central core of caudate nucleus (dog 62777). A degenerated dendritic process makes an asymmetrical synaptic contact with a normal terminal bouton (t). Normal axons of passage can be seen (a) and there is abundant extracellular space (ex). × 28,400.

Fig. 7. Central core of caudate nucleus (dog 62777). Several structures resembling enlarged, degenerating mitochondria (arrows) are apparently free in the extracellular space. A degenerating dendrite makes an asymmetrical synaptic contact with a normal terminal bouton (arrowheads). Despite the severe degeneration, a relatively normal dendrite (d), normal terminal boutons (t), and normal axons of passage (open arrows) can be seen. × 28,400.
Fig. 8. Caudate nucleus (dog 64015). Severe degeneration has reduced the neuropil to a structureless array of degenerated organelles and membrane-bound aggregates of amorphous debris (arrows). A relatively normal axon can be seen (arrowhead). × 4,500.

Fig. 9. A cell body with the morphological characteristics of a neuron persists in the degenerated caudate nucleus of dog 64015. × 8,300.

The following description is a composite of the progression of lesions in the molecular, Purkinje cell, and granule cell layers of the cerebellar cortex in all dogs. Lesions in specific dogs will be mentioned only where noteworthy.

Initial lesions were detected in Purkinje cell dendrites. In the main stem dendrites of a few Purkinje cells in dog 70352, the cytoplasm contained stacks of blunt-ended cisternae with up to 15 such cisternae in some areas (Fig. 10). Some of the dendrites were electron-dense but other organelles were not remarkable except that rough endoplasmic reticulum was not discernible. The earliest evidence of frank degenerative changes occurred in Purkinje cell dendrites. Affected dendrites of these cells, identified by their well-developed hypolemmal cisternae, had dense aggregations of mitochondria (Figs. 11, 12). These mitochondria were enlarged compared to those of surrounding normal axons and had an electron-dense granular matrix with numerous cristae. As the lesions progressed, there were many electron-dense, contracted dendritic processes scattered throughout the molecular layer that contained degenerated mitochondria and which possessed well-developed hypolemmal cisternae. In some instances, no identifying hypolemmal cisternae could be detected due to the extreme electron density of the dendrites. The degenerating dendrites were surrounded by morphologically normal axons and dendrites of other cell types, i.e. basket and stellate cells and axons of climbing and parallel fibers. Further progression of the lesions resulted in the disappearance of Purkinje cell dendrites and large areas with haphazardly arranged membranous profiles. This change gave the molecular layer a disorganized spongy appearance. As the degeneration of Purkinje cell dendrites progressed through the above described changes, radiating astrocytic processes...
Fig. 10. Molecular layer of cerebellar cortex (dog 70352). A main stem dendrite of a Purkinje cell contains many stacked cisternae. No well-developed rough endoplasmic reticulum is discernible. \( \times 10,500 \).

orientated perpendicular to the meningeal surface became increasingly more prominent. During the later stages, there was focal degeneration of myelinated axons in the molecular layer.

Minor alterations were detected in Purkinje cell bodies. Occasional cell bodies in dog 70352 contained stacked cisternae as previously described. Other cell bodies were either morphologically normal or were unrecognizable depending on the area of the cerebellar cortex examined and the stage of the disease. Cell bodies in the active process of degeneration were not detected. During the early stages, there was severe depletion of Purkinje cells in the vermis and paramedian lobule while cell bodies were more numerous in the lateral hemispheres. In the later stages, Purkinje cells were either totally absent or severely depleted throughout all parts of the cerebellar cortex.

Degenerative changes also occurred in the granule cell layer but these were less prominent than in the outer layers of the cortex. Occasional degenerated Purkinje cell axons were encountered during the early stages. The degenerated axons contained membranous bodies, discrete organelles, and were electron-dense but myelin sheaths remained intact. As the disease progressed to the later stages, granule cells became shrunken, contracted, and electron-dense.

**DISCUSSION**

The lesions in both the caudate nucleus and cerebellar cortex showed a similar progressive pattern of development during the active stages of the disease that eventually led to widespread neuronal degeneration in these areas. However, the initial occurrence of cytoplasmic lipid and amorphous osmiophilic intramitochondrial inclusions in neurons of the caudate nucleus, the latter also being compatible with
lipid or lipoprotein (6), was not a feature of the cerebellar lesions. No explanation could be given for the occurrence of these inclusions and their presence could not be correlated with the ensuing neuronal degeneration during the active stages of the disease. Neurons bearing these inclusions were otherwise normal, so these lesions may be insignificant. The stacked cisternae that occurred in Purkinje cell bodies and dendrites were not detected in caudate neurons. The significance of this alteration also could not be determined. Since no rough endoplasmic reticulum was demonstrable in the surrounding cytoplasm, it might be concluded that these cisternae developed from this organelle but this could not be confirmed. Lamellar bodies with a roughly similar morphology to these stacked cisternae have been reported following intracerebellar kainic acid injection (7) but they are almost identical to the bodies described in some cases of olivopontocerebellar degeneration (8). The most striking and consistent lesion in dendrites of both caudate neurons and Purkinje cells during the early stages of active degeneration was mitochondrial enlargement. This change was considered to represent mitochondrial hypertrophy and, as such, may have reflected an increased functional demand on these neurons. Similar changes have been reported in cardiac, skeletal and uterine muscle with various conditions in which increased functional demand is placed on these tissues (6).

The neuronal lesions in the caudate nucleus and cerebellar cortex were specific for intrinsic neurons in both areas of the brain. Survival of terminal boutons and axons was striking, even though there was almost total depletion of dendritic processes. The few degenerated axons that were encountered were interpreted as those
of intrinsic caudate neurons and Purkinje cells while surviving axons were considered axons of passage. The severe degeneration of intrinsic neurons, with sparing of axons of passage and terminal boutons of neurons originating outside the affected area, indicated a mechanism that was specific for neurons in the affected areas of the brain. From a pathogenetic standpoint, the similarity in the character and progression of lesions in the caudate nucleus and cerebellar cortex would suggest a common mechanism for the neuronal degeneration. Experimental animal models of intrinsic neuron-specific, axon-sparing lesions may indicate possible mechanisms for the neuronal degeneration in this canine disease.

Experimental evidence has indicated that glutamate, the first such animal model, is an excitatory neurotransmitter in the corticostratial (9–12) and granule cell (13–15) pathways of the caudate nucleus and cerebellar cortex, respectively. In several species, glutamate may cause neuronal degeneration by an excitotoxic mechanism when present at higher than physiologic levels while axons of passage are spared (16–20). Injection of kainic acid, a potent neuroexcitatory analog of glutamate, into regions of the brain that receive glutaminergic innervation causes neuronal degeneration by an excitotoxic mechanism similar to the one proposed for glutamate (21). Ultrastructurally, intrastriatal kainic acid injection results in degeneration of intrinsic neurons while axons of passage and terminal boutons are spared (22–26). The neuronal degeneration is characterized by clumping of Nissl substance (25), vacuolization of perikarya and dendrites (24, 26) and dilatation of endoplasmic reticulum (24, 26) with eventual pyknosis (25) and loss of virtually all intrinsic neurons (22–25). Intact terminal boutons making synaptic contacts with degenerated postsynaptic elements are prominent (22–25). In the cerebellum, the Purkinje, Golgi, basket, and stellate cells are sensitive to the neurotoxic effects of kainic acid (7, 13). The neuronal degeneration here is characterized by dilatation of the endoplasmic reticulum and extreme electron density with vacuolization of the cytoplasm (7, 13). Collapse of Purkinje cell dendrites occurs. Eventually, there is destruction and dissolution of the cells. Remnants of Purkinje cell dendritic spines attached to intact parallel fiber terminals persist for some time after the acute degeneration and lamellar bodies occurred in Purkinje cell perikarya and dendrites (7).

Based on the glutaminergic nature of the corticostratial and granule cell pathways and the neurotoxic potential of glutamate, a mechanism for the neurodegeneration in this canine disease has been proposed that is based on an alteration in these glutaminergic pathways (2). Apart from the specificity for intrinsic neurons and the axon-sparing nature of the lesions in both the naturally occurring and experimental diseases, there are important differences in the lesions at the ultrastructural level that should be considered. In the canine disease, changes regarded as mitochondrial hypertrophy were prominent in both the caudate nucleus and cerebellum. No such lesions have been described with the kainic acid model, but, in contrast, vacuolization and dilatation of the endoplasmic reticulum are consistent features. In addition, not all neurons in the caudate nucleus are affected simultaneously in the canine disease while virtually all neurons in the kainic acid model are affected. Furthermore, only Purkinje cells are involved to any great extent in the canine disease while the kainic acid injection affects not only the Purkinje but Golgi, basket, and stellate cells as well. Many factors could conceivably be involved which would account for these discrepancies, but these are conjectural. First, the kainic acid injection is an acute insult while the course of the canine disease is more protracted. The mitochondrial hypertrophy in degenerating neurons could be a compensatory response, reflecting an increased functional demand due to glutamate-induced persistent membrane.
depolarization, while in the kainic acid model, such hypertrophy would not have time to occur. Second, the probable heterogeneity of amino acid receptors throughout the central nervous system makes it impossible to assume the total destruction of all neuron types by a specific neurotoxic amino acid but, large doses of neurotoxic amino acids may override any receptor selectivity (16). This, then, could account for the fact that not all neurons in the caudate nucleus are affected simultaneously, or for certain neurons in the cerebellum, i.e. Golgi, basket, and stellate cells, not being involved in the canine disease. Finally, the convulsive activity following intracerebral injections of excitotoxic compounds (16) may introduce variables related to the enhanced neuronal activity or to the systemic alterations that commonly accompany the convulsive state.

Two other compounds which produce neuron-specific, axon-sparing lesions following intracerebral injection may indicate the importance of endogenous toxic metabolites in neurodegenerative diseases. Following intrastriatal injection of either quinolinic acid, a tryptophan metabolite (27) or L-pyroglutamic acid, an intermediate in the gamma-glutamyl cycle (28), there is dendritic (27, 28) or mitochondrial (28) swelling with resultant degeneration of postsynaptic elements while presynaptic terminals and axons remain intact (27, 28). Again, mitochondrial changes described in the canine disease have not been reported with these two experimental models. The same arguments presented in the kainic acid model, i.e. acute versus protected disease, would be applicable here as well.

This canine disease may prove to be an exciting animal model with which to study the relationship of endogenous neurotoxins to neurodegenerative disease. An excitotoxic mechanism of neuronal degeneration similar to the one proposed for this canine disease (2) has been implicated in three human neurodegenerative diseases with primary involvement of the caudate nucleus. In two of them, Huntington's disease (21, 22, 29) and infantile bilateral striatal necrosis (30), a direct effect of glutamate has been proposed as one of the mechanisms that might be involved in the neuronal degeneration. With the third, glutaric acidemia, a defect in the metabololism of glutaryl-CoA, and possible glutamyl-CoA, leads to excessive production of glutaric acid which is a potential structural analog of glutamate (31). In addition, roles for both quinolinic acid and L-pyroglutamic acid have been proposed for the neurodegeneration in Huntington's disease (27, 28).

REFERENCES


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