Motor neurone disease and animal models

Introduction

Over the past several years, remarkable progress has been made in understanding the biological substrates of motor neurone disease (MND) (Kuncl et al. 1992, Smith 1992, Williams & Windebank 1993). Genetic factors in the aetiology of MND have been identified, animal models have been developed and characterized, hypothetical pathogenetic mechanisms have been proposed and are being tested and promising therapeutic approaches are being explored. The investigation of MND is now one of the most exciting and challenging areas of research and provides a superb illustration of the dynamic interactions between clinical and basic research in neuroscience.

Key words: amyotrophic lateral sclerosis, neurofibrillary pathology, neurotrophins, SOD1 mutations, transgenic mice

Human motor neurone disease

Clinical syndromes

Human MND, including amyotrophic lateral sclerosis (ALS), are characterized by paralysis and a variety of other motor signs. The distinct clinical syndromes (i.e. inherited upper vs lower motor neurone signs) are the result of selective degeneration of subsets of motor nerve cells (Kuncl et al. 1992, Price et al. 1992b, Harding 1993, Williams & Windebank 1993). Weakness/muscle atrophy is attributed to large α-motor neurones of the brainstem and spinal cord, whereas spasticity, hyperreflexia, and extensor plantar signs result from lesions of upper motor neurones (Brownell et al. 1970, Delisle & Carpenter 1984, Tandan & Bradley 1985, Banker 1986, Hirano, 1991, Kuncl et al. 1992, Oppenheimer & Esiri 1992, Price et al. 1992a, Harding 1993, Williams & Windebank 1993). With a world-wide prevalence of >4–6/100,000, classical ALS is characterized by the presence of all of these signs, and electrodiagnostic studies disclose fibrillations, fasciculations, and giant polyphasic potentials; muscle biopsies demonstrate denervation atrophy. The spinal muscular atrophies (SMA) of infancy and childhood, the second most common form of autosomal recessive disease (1:6000 new births) (Harding 1993, Williams & Windebank 1993, Melki et al. 1994), are classified on the basis of severity into type I (Werdnig-Hoffman disease), type II, or type III (Kugelberg-Welander disease). Kennedy’s disease, an adult-onset X-linked recessive bulbo-SMA (Fischbeck et al. 1991, Kuncl et al. 1992, Matsaura et al. 1992), occurs in males and is characterized by progressive limb weakness and difficulty in swallowing.

Aetiological factors

Age is a risk factor in the expression of these diseases. Classical ALS usually begins in late life (Williams & Windebank 1993), whereas SMA occurs in infancy and childhood (Hamida & Hentati 1991, Hausmanowa-Petrusewicz 1991, Harding 1993). Approximately 10% of cases of adult-onset ALS are familial and show autosomal dominant inheritance associated with age-dependent penetrance (Siddique et al. 1989, 1991) In families with early-onset familial ALS (FALS), missense mutations have been discovered in superoxide dismutase 1 (SOD1) (Deng et al. 1993, Rosen et al. 1993), which is encoded by an ~15-kD gene comprised of five exons on chromosome 21 (Rosen et al. 1993) SOD1 is a member of a family of metalloenzymes characterized by an ability to dismutate O$_2^-$ (Fridovich 1986) (i.e., to catalyse the conversion of O$_2$, the product of spontaneous and enzyme-catalysed oxidation, into H$_2$O$_2$ and O$_2$). Behaving as a reductant or oxidant, O$_2^-$ gives rise to reactive molecules that can injure cells by a variety of mechanisms (Imlay & Linn 1988, Halliwell 1991, Stadtman 1991). The majority of cases of childhood/juvenile SMA shows linkage to DNA markers on chromosome 5q13 (Melki et al. 1990), and recent studies have demonstrated inherited and de novo deletions in these patients with SMA (Melki et al. 1994). Kennedy’s disease is caused by an expansion of a tandem CAG trinucleotide repeat in exon 1 of the androgen receptor gene (X q11–12) (La Spada et al. 1991, Caskey et al. 1992, Doyu et al. 1992), differences in repeat length correlate with the onset of disease.

Neuropathology

Various MND selectively involve subsets of motor nerve cells. Affected neurones frequently show accumulations of 10-nm neurofilaments (NF) in axons and cell bodies and a
variety of perikaryal inclusions as well as phosphorylated NF and ubiquitin immunoreactivities and neurofilamentous swellings of proximal axons (Hirano 1991, Chou 1992, Hirano & Kato 1992, Lee et al. 1994a). The calibers of distal axons are reduced, and there is evidence of axonal Wallerian degeneration (Delisle & Carpenter 1984, Banker 1986, Manetto et al. 1988, Munoz et al. 1988, Sasaki et al. 1988, Lowe et al. 1993, Harding 1993, Williams & Windebank 1993). It is not known if there are accompanying changes in levels of RNA, as RNA blotting reveals normal levels of mRNA of the 68-kD polypeptide neurofilament subunit (NF-L), but in situ hybridization shows that levels of NF-L and poly A mRNA are decreased in motor neurones (Bergeron et al. 1994). Eventually, these cells die. End-stage disease is characterized by reduced numbers of motor neurones in brainstem nuclei/spinal cord, loss of large pyramidal neurones in motor cortex, and degeneration of peripheral motor axons and axons in corticospinal tracts (with denervation of target fields).

The mechanisms of selective vulnerability, cytoskeletal dysfunction, and cell death involving at-risk neuronal populations are not yet well understood. Dysfunction/death of motor neurones has been attributed to: genetic abnormalities (i.e., mutations of the SOD1 and androgen receptor genes) (Fischbeck et al. 1991, La Spada et al. 1991, Caskey et al. 1992, Dooy et al. 1992, Matsuura et al. 1992, Rosen et al. 1993); oxidative injury (Rosen et al. 1993); nitration of protein (Beckman et al. 1993); excitotoxicity, possibly mediated by glutamate receptors (Monaghan et al. 1989, Tsai et al. 1991, Rothstein et al. 1992, 1993, McNamara & Fridovich 1993, Lipton & Rosengburg 1994, Rothstein et al. 1994); calcium influx (Lipton et al. 1994); immunological mechanisms (Appel 1993), and perturbations in the neuronal cytoskeleton (Cork et al. 1979, 1982, Brady 1993, Côté et al. 1993, Xu et al. 1993). Because autopsy studies of individuals with ALS almost invariably show end-stage disease, it is difficult to reconstruct the evolution and mechanisms of cellular dysfunction and death in these cases. Therefore, investigators have turned increasingly to studies of animal models (Table 1): to define the aetiologies of disease; to characterize the evolution of pathology; to analyse, using a variety of neurobiological strategies, pathogenetic mechanisms; and to test novel therapies for these disorders.

### Animal models of motor neurone disease

Over the years, investigators have studied a variety of animal models involving motor neurones, including the neurofilamentous axonopathy caused by intoxication with β,β′-iminodipropionitrile ([IDPN]); Brittany spaniels with hereditary Canine Spinal Muscular Atrophy (HCSMA); several spontaneously occurring marine models, including progressive motor neuronopathy (pmn) and murine motor neurone degeneration (mnd); transgenic mice that overexpress normal or mutated NF genes; transgenic mice expressing SOD1 mutations; several gene knockout mice created by gene-targeting strategies; and axotomy-induced retrograde degeneration.

### IDPN-induced neurofilamentous axonopathy

The systemic administration of IDPN causes weakness, maloriented arrays of NF accumulated in swollen proximal motor axons, and atrophic distal axons (Griffin et al. 1978, Clark et al. 1980, Gold et al. 1986). Although these IDPN-induced abnormalities are not selective for motor neurones, pathology in the ventral horn and motor nerves resembles that occurring in cases of ALS and in some aged individuals (Clark et al. 1984). IDPN impairs the slow transport of NF proteins, probably because the toxins appear to dissociate NF from microtubules (Griffin et al. 1978, 1983a, b). Similar alterations in NF transport are presumed to occur in some cases of ALS.

### Hereditary canine spinal muscular atrophy (HCSMA)

This autosomal dominant disease in Brittany spaniels is characterized by weakness and atrophy of skeletal muscles (Cork et al. 1979, Lorenz et al. 1979, Sack, et al. 1984, Cork 1991) with relative sparing of eye movements and sphincters. Electromyograms and muscle biopsies show patterns consistent with denervation atrophy. Mating affected-to-affected dogs produces a homozygote with accelerated disease manifest as tetraplegia by 3–4 months of age. Heterozygous animals show two patterns: intermediate phenotype dogs become weak at ~6 months and are severely paralysed at 2–3 years; and dogs with the chronic phenotype develop mild weakness within the first 2 years of life and live for >6 years. To date, HCSMA has not been linked to any chromosomal locus. All dogs with HCSMA phenotypes develop neurofilamentous swellings in proximal axons of motor neurones (Cork et al. 1979, 1982); many swollen axons are denuded of myelin sheaths. Some neurones show increased NF immunoreactivity in perikarya (Cork 1991). Cytoskeletal pathology, very similar to that occurring in cases of ALS (Delisle & Carpenter 1984, Hirano 1991), is attributed to impairments in the transport of the NF triplet proteins (and possibly tubulin) (Griffin et al. 1982). Perikaryal size and motor axonal diameters are reduced—abnormalities interpreted to reflect, in part, growth arrest and axonal atrophy.

**Table 1.** Selected models of diseases involving motor neurones

<table>
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<th>Disease Model</th>
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<td><strong>IDPN-induced neurofilamentous axonopathy</strong></td>
<td>- SOD1 mutations</td>
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<td><strong>HCSMA</strong></td>
<td>- Transgenic mice overexpressing the NF genes or expressing deletions or point mutations in NF genes</td>
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<td><strong>pmn</strong></td>
<td>- Knockout mice: BDNF; trkB; CNTF</td>
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(Cork et al. 1989a, b). Although these dogs are profoundly weak, motor neurones do not degenerate through the course of illness, and it has been suggested that some weaknesses may be caused by the failure of swollen, NF-filled axons. HCSMA is an excellent model of MND.

**Progressive motor neuronopathy (pmn)**

This disease was discovered as a spontaneously appearing autosomal recessive disorder (Schmalbruch et al. 1991). Homozygous mice develop hindlimb paralysis followed by quadriaparesis and ultimately die 6–7 weeks after birth (Schmalbruch et al. 1991). Some spinal motor neurones show mild chromatolysis, motor axon degeneration (particularly distally), and neurogenic atrophy of muscle. As illustrated by detailed studies of phrenic nerves, the disease appears to be a dying-back process with distal axons degenerating and relative preservation of proximal axons and cell bodies. Trophic therapeutic approaches to this model have been based on the idea that ciliary neurotrophic factor (CNTF) is a trophic factor for motor neurones (see below). It has been suggested that the administration of CNTF ameliorates aspects of the dying-back process (Sendtner et al. 1992b). The effects of CNTF on other parameters of motor neurones are uncertain, and this report (Sendtner et al. 1992b) has generated some controversy (Vrbová et al. 1992, Sendtner et al. 1992c). This murine disease (Schmalbruch et al. 1991) is clearly distinct from that occurring in Wobbler mice and mnd mice (see below) (Messer & Flaherty 1986, Bronson et al. 1993, Pardo et al. 1994).

**Murine motor neurone degeneration (mnd)**

Originally recognized as a spontaneous adult-onset neurological disease that occurs in C57BL/6 mice of both sexes (Messer & Flaherty 1986), mnd now appears to be an autosomal dominant that maps to the proximal arm of chromosome 8 (Messer et al. 1992b). Mice develop motor abnormalities, initially in the hindlimbs; eventually, animals show greatly reduced spontaneous movement and die before 1 year of age. Because animals exhibited reduced mobility and because the original studies focusing on motor neurones were interpreted to show selective pathology of these nerve cells (Messer et al. 1987, Messer & Plummer 1993, Callahan et al. 1991, Mazurkiewicz 1991), the disease was proposed initially to be a model of ALS (Messer 1992a). However, recent investigations (Bronson et al. 1993, Pardo et al. 1994) have shown that these mice exhibit widespread abnormalities of neurones, including the accumulation of lipofuscin and ATP synthase, subunit 9(c) (Bronson et al. 1993, Pardo et al. 1994). The presence of these abnormalities, characteristic of neuronal ceroid lipofuscinosis (Palmer et al. 1992), suggests that mnd is a murine form of neuronal ceroid lipofuscinosis. This murine disease should be of great value for studies of the genetic basis of neuronal ceroid lipofuscinosis, for investigations of the mechanisms of neuronal dysfunction/death in these types of metabolic disorders, and for testing novel treatments of this illness (Pardo et al. 1994).

**Transgenic mice with altered NF expression**

NF are assembled from three subunits, NF-L (68 kD), NF-M (95 kD), and NF-H (115 kD); in vivo, NF are formed as obligate heteropolymers (Lee et al. 1993, Lee & Cleveland 1994a). In the normal setting, these intermediate filaments are the primary determinant of axonal caliber (Hoffman et al. 1985, 1987, Eyer & Peterson 1994, Lee & Cleveland 1994a). Because the abnormal accumulation of NF in the perikaryon and proximal axons is the most frequent early pathological hallmark of ALS, three sets of transgenic mice have been constructed to examine whether increased NF content or the expression of NF mutation can be a direct cause of mnd. Although up to twofold increases in the expression of wild-type mouse NF-L did not cause an overt phenotype (Monteiro et al. 1990), additional increases in expression lead to prominent perikaryal and axonal swellings comprised of bundles of closely packed NF (Xu et al. 1993). Normal axonal function is disrupted as revealed by severe denervation-induced skeletal muscle atrophy. Animals die within the third or fourth week of age. Doubling NF-H content by expression of a wild-type human NF-H transgene resulted in an almost-identical pathology with the important exceptions that the age of onset was later (4–5 months of age) and that there was a more gradual progression of disease. Both examples clearly demonstrate that neurofilamentous accumulations can cause motor neurone dysfunction and muscle atrophy. Transgenic mice show very little axonal degeneration—an important difference as compared with ALS. Significantly, mutations in the NF gene can directly cause MND, including death of spinal motor neurones. Transgenic animals that express half of the normal level of mouse NF-L subunit with a single amino acid substitution show selective death of nerve cells (Lee et al. 1994b). In different lines, age of onset varies between 3 weeks and 3 months and is dependent on the level of the mutant NF-L subunit. Animals show prominent axonal and perikaryal swellings comprised of disorganized bundles of filaments. Together, these studies of transgenic mice indicate that primary alterations in NF can cause neurodegenerative disorders that resemble those occurring in cases of ALS and that accumulations of NF can play important roles in the cascade of events that lead to motor neurone disease (Lee et al. 1994a). Although a pair of mutations in the NF-H tail domain has been found in five patients with sporadic ALS, it is not proven that the human disease derives from primary mutations in NF.

**SOD1 transgenic mice**

Recent discoveries that mutations in the Cu/Zn SOD1 gene are linked to FALS (Deng et al. 1993, Rosen et al.
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1993) have spurred the construction of transgenic mice that express mutant SOD1. Gurney et al. (1994) demonstrated that mice with the G93A mutation develop weakness of the limbs and die by 5–6 months of age. Animals with the G93A mutation show loss of motor neurones, Wallerian degeneration in the ventral roots, and denervation atrophy. A similar phenotype occurs in mice with the G37R mutation (Wong et al., unpublished observations). G37R mice develop striking axonal swellings and hindlimb weakness at 3.5–4.0 months of age; death usually occurs 4 weeks after the onset of neurological disease. Moreover, examination of the four lines of mice with different levels of transgene expression suggests that the onset is related to transgene copy number and levels of transgenic product. The initial expectations that SOD1 mutations could cause disease by lowering SOD1 activity (Deng et al. 1993) appear not to be correct, at least in these transgenic models. In G93A mice, SOD1 activities are substantially elevated (2–3-fold) (Gurney et al., 1994), and assays of the specific activity of the G37R polypeptide reveal the mutation enzyme to be more active than the wild type (Borchelt et al., 1994). In a different strain of mice, the overexpression of wild-type human SOD1 yielded abnormalities of nerve terminals in the distal muscles and tongue (Avraham et al., 1988, 1991). Whether defects resulting from over-expression of wild-type SOD1 are confirmed in additional mouse lines is not known, but, in mice from the same genetic background as affected G37R mice, no overt phenotype is seen even when SOD1 activity is raised fivefold by the presence of a wild-type human SOD1 transgene. These findings are consistent with the concept that SOD1 mutations lead to a gain of some dominant adverse function rather than to a loss of SOD1 activity. Moreover, the distribution of SOD1 immunoreactivity does not parallel cell vulnerability in FALS, although SOD1 is abundant in perikarya, dendrites, and axons of motor neurones (Pardo et al., unpublished observations). CA2-CA4 hippocampal neurones, which are unaffected in FALS, also have high levels of SOD1. Thus, the present evidence suggests that motor neurones degenerate because SOD1 mutations are associated with aberrant gain-of-function activities; these activities may be unrelated to conversion of superoxide to hydrogen peroxide (Borchelt et al., 1994). Mice that express FALS-linked SOD1 mutations can now be used to test the roles of these mutations in disease, to establish the characteristics and evolution of neuronal abnormalities associated with these mutations, to clarify the mechanisms of motor neurone vulnerability and dysfunction/death, and to test novel therapies.

**BDNF and trkB knockout mice**

With the cloning of neurotrophic factors and their receptors, which influence specific populations of cells, investigators have used gene-targeting strategies to ablate genes coding for these factors/receptors and to examine the consequences in null (−/−) mice (Klein et al., 1993, Masu et al., 1993, Davies 1994, Jones et al., 1994, Snider 1994). Several factors/receptors, including trkB, brain derived neurotrophic factors (BDNF), and CNTF are proposed to be important in the biology of motor neurones. Targeted disruption of the BDNF gene (Jones et al., 1994) results in homozygous mutants that usually die within 2 days of birth. These animals have substantially reduced numbers of cranial and spinal sensory neurones, but motor neurones appear normal. In contrast, a germline mutation in the tyrosine kinase catalytic domain of the trkB gene eliminates the expression of gp 145 trkB, resulting in homozygous mice that do not feed well and usually die in the early postnatal period (Klein et al., 1993). These animals have an ~35% reduction in the number of motor neurones (70% in the facial nucleus; 35% in lumbar spinal cord) as well as abnormalities in a variety of other subsets of cells in the peripheral and central nervous system. A comparison of these two mutations suggests that other factors influencing motor neurones (e.g. NT-4/5) may be able to compensate for the BDNF null state in trkB-expressing motor neurones (Davies 1994).

CNTF is expressed normally in Schwann cells and some astrocytes. CNTF (−/−) animals show some evidence of atrophy and loss of motor neurones accompanied by small reductions in muscle strength with aging animals (Masu et al., 1993). Thus, CNTF does not appear to be necessary for the development of motor neurones but may play some role in the maintenance of these cells in postnatal life (Masu et al., 1993).

**Axotomy-induced retrograde degeneration of neurones**

Transsection of motor neurones, a model of axonal injury showing certain features relevant to MND, interrupts fast anterograde and retrograde transport (Griffin et al., 1977), leading to the accumulation of membranous elements and the transport of endogenous and exogenous proteins at the proximal and distal stumps of axons (Nissl 1892, Bodian & Mellors 1945, Price & Griffin 1977, Griffin et al., 1976, 1977, 1981, Carroll et al., 1978, Price et al., 1984, Koo et al., 1990, Koliatsos et al., 1991b). Axotomized neurones show: chromatolysis (Price & Porter 1972, Price et al., 1984); alterations in levels of specific mRNA/proteins, such as NF proteins, peripherin, and tubulin (Hoffman et al., 1984, 1985, Koo et al., 1988, Muma et al., 1990, Troy et al., 1990); aberrant distributions of cytoskeletal proteins (i.e. phosphorylated NF in perikarya) (Rosenfeld et al., 1987); and changes in the transport of some of these proteins (Hoffman et al., 1985, 1987; Koo et al., 1988, Muma et al., 1990). After axotomy, levels of NF gene expression are reduced (Hoffman et al., 1987), whereas levels of β-gal-tubulin show little or no decrease (Troy et al., 1990). The amounts of NF proteins entering axons are decreased, and a wave of reduced axonal caliber moves down the axon at the rate of transport of NF proteins (Hoffman et al., 1985). Eventually, the synthesis of NF proteins returns to normal, and axonal caliber is restored (Hoffman et al., 1985, 1987).
observations consistent with the concept that NF are one determinant of axonal caliber (Hoffman et al. 1984, 1985). After axotomy, markers for neurotransmitter-related components decrease (Koo et al. 1988, Koliatsos et al. 1991b), and, in adult motor neurons, p75<sup>NGFR</sup> (nerve growth factor receptor) expression increases (Koliatsos et al. 1991b). The p75<sup>NGFR</sup> immunoreactivity is present exclusively in axotomized neurons, as verified by the colocalization of p75<sup>NGFR</sup> immunoreactivity with a fluorescent retrograde tracer injected at the crush site. When motor neurons reinnervate targets, p75<sup>NGFR</sup> immunoreactivity disappears, indicating that p75<sup>NGFR</sup> expression is linked closely with disconnection of cells from the target (Koliatsos et al. 1991b).

More recently, axotomy models have begun to be used to study processes that lead to cell death and to test therapies that preserve cell phenotype and promote cell survival (Koliatsos et al. 1990, 1991a, 1994, Sendtner et al. 1990, 1992a, Yan et al. 1992). For example, when the facial or sciatic nerves of neonatal rats or the L4-5 ventral roots of adult rats are transected, motor neurons undergo retrograde degeneration (Sendtner et al. 1990, 1992a, Yan et al. 1992, Koliatsos et al. 1993, 1994). Cytokines were the first molecules to be tested in this model (Sendtner et al. 1990), and early results suggest that CNTF ameliorates cell loss in the facial nucleus (Sendtner et al. 1990); however, other investigators have not been able to demonstrate a robust effect in this setting (Snider et al. 1992, Clatterbuck et al. 1994). Recently, several lines of evidence have been presented to show that BDNF, but not NGF or NT-3, is a trophic factor for motor neurons (Koliatsos et al. 1993). BDNF is expressed in the local environment and in muscle targets of motor neurons, and muscle expression is up-regulated by denervation; this neurotrophin is present in skeletal muscles; and, in the facial nerve transection paradigm (Koliatsos et al. 1993), BDNF is transported selectively to α-motor neurons from skeletal muscles; and, in the facial nerve axotomy model, gelfoam pads containing human recombinant BDNF apposed to the proximal stump reduced cell death to 20% in the vehicle-treated group (Koliatsos et al. 1993, 1994). These findings, consistent with work published recently from other laboratories (Oppenheim et al. 1992, Sendtner et al. 1992a, Yan et al. 1992), suggest that BDNF has a trophic effect on motor neurons. More recently, NT-4/5 has been shown to have similar effects on motor neurons injured in the facial nerve transaction paradigm (Koliatsos et al. 1994). The actions of BDNF and NT-4/5 on motor neurons and the presence of these high-affinity receptors in these cells raise the possibility that the administration of neurotrophins may be useful in treating transgenic mice with FALS-related SOD1 mutations.

**Conclusions**

This review outlines recent progress in understanding the clinical features, genetics, and neuropathology of human MND and details the ways in which animal models are being used to examine the roles of specific aetiological factors, to test pathogenetic mechanisms, and to evaluate potential therapies relevant to MND.

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**References**


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