Alterations in Nitrogen Monoxide–Synthesizing Cortical Neurons in Amyotrophic Lateral Sclerosis with Dementia

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Abstract. Cognitive impairment in the absence of lesions indicative of Alzheimer’s disease and other dementing conditions has long been recognized in a subgroup of patients with motor neuron disease (MND), including amyotrophic lateral sclerosis. However, the mechanisms underlying this cognitive deterioration and its relationship with the relatively selective involvement of motor neurons remains elusive. We used histo- and immuno-cytochemical labeling methods to study the nitric oxide (NO; i.e., nitric oxide synthase (NOS))/NADPH diaphorase-containing neurons (NOSN) in three patients with MND and dementia (MND + D), two patients with MND without dementia, and 19 controls that included patients with Alzheimer and non-Alzheimer dementias. Patients with MND + D, but not those with MND without dementia, exhibit numerous dystrophic perikarya and neurites throughout all sensory, motor, association, and limbic neocortices examined. Interestingly, affected NOSN appear to correspond to some subtypes (smooth stellate and spiny neurons), while other neurons containing the same molecular phenotype (such as layer I local circuit neurons and layer II granule cells) are either spared or significantly less affected. These observations indicate that cognitive impairment and dementia in MND may be due, at least in part, to a pancellular involvement of certain types of NOSN. Consequently, the elucidation of the factors that make NOSN vulnerable in MND, and the prevention or pharmacological palliation of their loss, may eventually help to prevent or ameliorate cognitive impairment in MND and may also shed some light on the nature of the insult that targets motor neurons.

Key Words: Amyotrophic lateral sclerosis; Dementia; Local circuit neurons; Motor neuron disease; NADPH diaphorase; Neocortex; Nitrogen monoxide.

INTRODUCTION

The motor neuron diseases (MND) are generally regarded as processes confined to the voluntary motor system, with progressive degeneration of the corticospinal tracts or alpha motor neurons, or both. It has been relatively easy to understand on the basis of the latter degeneration how these diseases result in the well-documented progressive muscular weakness and wasting (1). There are, however, multiple reports of associated dementia, parkinsonism and sensory changes indicating that the disease process is not entirely selective to the voluntary motor system (1–17). We are most interested in the precise substrate of cognitive and behavioral impairment in MND, because it would seem to reflect the involvement of multiple regions of the brain, as opposed to the comparatively more circumscribed involvement that may underlie the motor and sensory manifestations in this condition. This involvement of multiple areas of the cerebral cortex may not be indiscriminate or non-

selective to the large numbers of anatomically, functionally and molecularly distinct circuits distributed throughout the brain, as suggested by the findings presented here (see below).

Dementia in patients with MND can sometimes be associated with co-existing conditions, such as Alzheimer’s disease (5, 16), spongiform encephalopathy, and Pick’s disease (5, 8). However, no concomitant conditions can be demonstrated in many such patients with dementia (1–17). In these latter cases, it may be that either the MND and the dementia are caused by the same process or MND with and without dementia are nosologically different entities. In any event, the precise substrate of cognitive impairment—which can precede all other symptoms in these cases (2)—remains a mystery.

There are a few reports indicating that the number of large and medium-sized pyramidal neurons is reduced in the cortex, which presumably results also in the vacuolation or "spongiform changes" reported in the superficial layers of the frontal and temporal lobes (14). This observation might imply that smaller neurons such as nonpyramidal neurons are comparatively unaffected. However, this interpretation relies heavily on inherent difficulties to assess quantitatively small nonpyramidal neurons (in contrast with the larger pyramidal neurons) in a rigorous fashion in postmortem human material, and the fact is that there is no conclusive evidence that smaller neurons are affected or spared in patients with MND. Even if one takes the reported loss of large pyramidal neurons at face value, it is hard to understand why presumably non-motor neurons are affected in the temporal lobe, just as it is hard to accept that all pyramidal neurons
apparently lost throughout the frontal lobe must be corticospinal and corticobulbar neurons.

The present report is part of a series of studies on the role of anatomically, functionally, and molecularly unique populations of neurons in the forebrain in the cognitive deterioration associated with several neurodegenerative disorders (18–20). Here we present evidence that unique subpopulations of neurons that co-contain the syn- thase (NOS) for the newly identified neurotransmitter nitrogen monoxide (NO), and NADPH diaphorase—which some believe is identical with the synthase (21, 22)—appear to degenerate in MND with dementia (MND + D) but not in MND without dementia (hereinafter designated simply as MND). The involvement of NOS-containing neurons (NOSN) may thus underlie, at least in part, the cognitive impairment observed in some patients with MND. Although it is unknown at present whether additional abnormalities underlie MND + D, and in the absence of conditions that can coincide with MND (e.g., Alzheimer’s disease, spongiform encephalopathy), the panchoroidal and panlaminar involvement of NOSN we found could be sufficient by itself to produce cognitive deterioration. This hypothesis has important implications for the design of future strategies to palliate and to prevent cognitive deterioration in patients in which NOSN are affected.

MATERIALS AND METHODS

We used brains from 18 patients with various types of dementia and six controls. Five suffered from MND (three with dementia, two without dementia). Age-matched and younger control cases included patients that died of gunshot wounds, congestive heart failure and rheumatic heart disease (Table 1). Additional controls with dementia not associated with MND included patients with Alzheimer’s disease, dementia with cortical Lewy bodies (18), progressive supranuclear palsy (23), dementia lacking distinctive histologic features (24), and corticobasal degeneration (25). Tissues were obtained from the University of Miami’s Brain Endowment Bank and The University of Iowa Hospitals and Clinics and the Veterans Affairs Medical Center, Iowa City. A detailed protocol for this work was approved in advance by the institutional Human Subjects Review Committee.

Seven tissue blocks were dissected fresh from Brodmann’s areas 9, 24, 3, 4, 22, 7 and 17 (26) from each patient, using incisions perpendicular to the pial surface, such that the resulting blocks (about 1.5 × 1.5 × 2 cm) contained the entire thickness of the cortical grey and underlaying white matter. The blocks were then fixed in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB), pH 7.4, at 4°C, or 4% formal- dehyde, for 4–16 hours. These samples were then prepared for frozen sectioning in a sliding microtome, in a plane perpendicular to the major axis of cortical gyri of interest (transverse sectioning), or parallel to the pial surface (tangential sectioning), as described previously (20, 27).

Tissue sections from the above motor, sensory, limbic, and association neocortices were reacted histochemically for NADPH diaphorase as described recently (27) or labeled immunocytochemically with antibodies to NOS. Figure 1 illustrates the normal pattern of labeling that permits the identification of several different types of NOSN, which is indispensable to interpret the alterations in MND described in the present study. For immunolabeling, tissue blocks were cryoprotected in 30% sucrose in PB and sectioned frozen at 50 μm intervals in a sliding microtome. After rinsing in PB, sections were incubated in 1:1,000–5,000 dilutions of antisera to constitutive NOS (B 220-1; Eurodiagnostica, Malmö, Sweden; PA3-032, Affinity Bioreagents, Neshanic Station, NJ) in PB with 0.03% Triton X-100, for 24–72 hours at 4°C. Best results were obtained with the former antiserum at 1:5,000, using published immunocytochemical protocols (18). Controls included sections reacted without primary antibody, with primary antibodies to substances other than NOS, with no “bridge” (biotinylated) IgG, and without the avidin-biotin-peroxidase complex used to localize diaminobenzidine polymerization product (18–20). These controls failed to reveal labeling similar to the specific neuronal labeling obtained with the above antibodies. The best resolution of neuronal processes was obtained with the histochemical procedure for NADPH diaphorase, which was therefore used to prepare the preparations in the present report (Figs. 1, 3–5).

In addition to the above experimental procedures, tissue samples were taken from the aforementioned cortical areas and the hippocampus, thalamus, basal telencephalic nuclei, various regions of the brain stem, cerebellum and spinal cord. These were embedded in paraffin and processed for the full battery of histopathological methods we use for the differential diagnosis of neurodegenerative conditions. This included preparations according to the method of Bielschowsky, hematoxylin and eosin, luxol fast blue, Congo red, Holzer’s method and immunocytochemical labeling for glial fibrillary acidic protein.

Labeled NOSN were analyzed using computer-assisted photomontages of multiple microscopic focal planes (Figs. 3–5) and camera lucida drawings (Fig. 2). Computer-assisted photomontages were prepared from digitized images of multiple focal planes entered into a commercially available program (Adobe Photoshop, version 3.0.1, Adobe Systems, Inc., Mountain View, CA). Digitized images were obtained directly with a video camera attached to a light microscope or from 35 mm film with a scanner (Kodak 35 mm Rapid Film Scanner, Kodak, Rochester, NY). Normal or dystrophic elements of interest and in focus were then cut and pasted onto a single focal plane, yielding a virtually two-dimensional image of all relevant focal planes “collapsed” into one (Kuljis, report of technique in preparation).

All patients with MND, with and without dementia, as well as those with Alzheimer’s disease, dementia with cortical Lewy bodies, corticobasal degeneration, progressive supranuclear palsy, and the single case with cryptogenic dementia (Case F in Table 1) underwent extensive neuropsychologic testing, according to the protocol of the Benton Neuropsychology Laboratory (28).

RESULTS

All patients underwent neurological work-up, neuropsychological assessment, conventional histopathological examination of the brain, and cytochemical study with
### TABLE I

**Patients Examined**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
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<th>Autolysis (hours)</th>
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<td>A</td>
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<td>B</td>
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<td>G</td>
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MND + D: Motor neuron disease and dementia without Alzheimer's lesions or other superimposed dementing condition; MND: Motor neuron disease without dementia; CHF: Congestive heart failure; RHD: Rheumatic heart disease; GSW: Gunshot wound; PSP: Progressive supranuclear palsy; DCLB: Dementia with cortical Lewy bodies; CBD: Corticobasal degeneration; AD: Alzheimer's disease; N/A: Not applicable, patient was not demented.

*: Cryptogenic dementia, without Alzheimer's disease or other specific lesions; **: Multiple brain infarcts and alcoholism.

The term "autolysis" refers to the interval (in hours) between the death of the patient and fixation of the brain samples for this study.

Antibodies to NOS and histochemistry for NADPH diaphorase. This comprehensive assessment battery confirmed the clinical diagnosis of MND and ruled out additional dementing conditions in the five index patients.

**Neuropsychological Findings**

Neuropsychological testing revealed that three of the patients with MND (cases A–C, Table 1) suffered from deterioration in multiple areas of cognition, as well as of behavioral impairment, in addition to their motor symptoms. Two other patients with MND (cases D and E, Table 1), by contrast, did not exhibit cognitive deterioration in the same battery of tests that verified the clinical impression of cognitive impairment in cases A–C. Depression did not appear to be a factor influencing the performance of any of the above patients with MND. Similar examinations revealed unequivocal dementia in all patients with Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration and cortical Lewy bodies.

**Gross Examination and Histopathological Findings**

Patients with MND with and without dementia exhibited mild generalized brain atrophy roughly compatible with their age, except for case A. He exhibited moderate bifrontal atrophy and, in addition, had an uncomplicated aneurysm of the anterior communicating artery about 10 mm in diameter. This aneurysm had been discovered incidentally in the course of computed tomography for neurological work-up of the MND but no symptoms attributable to the aneurysm were ever recorded. No infarcts, tumors, malformations or other macroscopic structural lesions were present in the patients examined.

A battery of histopathological methods was used in sections from motor, sensory, limbic and association neocortices, basal telencephalic nuclei, thalamus, midbrain, pons, medulla, and at least the cervical spinal cord. These sections were stained with hematoxylin and eosin, Congo red, and thioflavin S, or immunopregnated with silver according to the modified method of Bielschowsky or the method of Campbell et al., or immunolabeled with antibodies/antisera to amyloid β/A4 protein and ubiquitin (20). This...
material failed to reveal lesions in a pattern and density compatible with current criteria for the diagnosis of Alzheimer's disease, Creutzfeldt-Jakob disease, or progressive supranuclear palsy in all our patients with MND. Thus, although occasional senile plaques and neurofibrillary tangles were found, these were sparse, as in controls without dementia of similar age. Similarly, Lewy-like bodies, or more than very occasional argyrophilic and neurofilament-positive inclusion bodies, and neuropil threads were not observed in patients with MND, whether with or without cognitive deterioration. The above occasional lesions were indistinguishable in number and appearance from those in age-matched controls.

Dystrophic NOSN Perikarya and Neurites in MND + D, but Not in MND Alone

Numerous profiles of grossly distorted, fragmented, balloononed and otherwise abnormal NADPH diaphorase/NOS-positive neurites and perikarya were consistently found in patients with MND and cognitive deterioration (Figs. 2, 3). Similar dystrophic neurites were not observed in patients with MND without cognitive impairment, or in controls without dementia (Fig. 1).

The dystrophic neurites could be visualized better with the histochemical method for NADPH diaphorase than with antibodies to NOS, perhaps because the former method tolerates better the inescapable postmortem autolysis and pre-labeling fixation necessitated in our material. Cell bodies, in our hands, were visualized about equally well with either method. In general, the quality of the histochemical preparations was adequate to discern enough detail to permit the cytological detection of newly recognized types of NOSN (27) and their dystrophic changes as reported here.

Caution was exercised to avoid confusing presumably dystrophic changes due to MND itself with the inescapable changes due to postmortem autolysis (29). Several approaches were used to minimize this difficulty, including: (1) cases with very short autolysis (i.e. up to 1 hour) were secured (cases A and E, Table 1); (2) a study of the range of cytological variability within normal human NOSN was conducted in anticipation to the present study (27), and (3) perfusion-fixed (i.e. devoid of postmortem

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Fig. 1 Photomicrographs of the main cytologically distinct types of NADPH diaphorase-positive neurons in the normal human brain. A: LCN in layer I (arrow points to the presumed axon). B: Layer II granule cells (shown at higher magnification in Figs. 4A, B). Arrowheads point to only some among the multiple neurons labeled in this layer. C: Smooth stellate neurons in layers II/III and V–VI and in the subcortical white matter. D: Densely spinous neurons situated predominantly in layers V–VI and in the white matter immediately below the cortical grey. Curved arrows indicate some of the dendritic spines in focus. Modified, with permission, from Fischer and Kujis (27).
NITROGEN MONOXIDE NEURONS IN ALS DEMENTIA

Fig. 2. Camera lucida drawing of the distribution of dystrophic neurites (black squares) and perikarya (asterisks) in one semitangential section of the primary auditory cortex, from case C. The continuous line represents the pial surface and the coarse broken line discontinuities in the tissue block. The fine broken line represents the interface between the grey and the white matter (WM). Note that both types of dystrophic elements are situated throughout all layers of the cortex and in the white matter underlying the cortical grey.

Autolysis (rodent, ovine and nonhuman primate brains were compared with the present human material (30; Kuljis, in preparation) in an additional effort to distinguish cytological changes attributable primarily to autolysis from actual pathology.

Clearly dystrophic neurites and perikarya were situated in all neocortical regions examined, whether sensory, motor, association, or limbic, and in all cortical layers. In addition, a substantial amount of both types of abnormal profiles were present in the white matter immediately under the cortical grey (Fig. 2), which is consistent with the observation that NOSN perikarya and neurites are present in densities higher than in the cortical grey proper at that level (27). Dystrophic elements consist principally of neurites (Fig. 3A–E, arrowheads) that are often impossible to identify unequivocally as dendrites vs axons, but we believe that both types of neurites contribute to these altered processes. The dystrophic elements consist of one or more clustered, deformed, fragmented, and irregularly shaped processes that virtually always resemble neurites (Fig. 3A–E). They exhibit abrupt changes in shape and diameter (when fundamentally cylindrical), with markedly thinned or distended portions, occupying multiple optical focal planes that traverse cytoarchitectonic layers and inter-areal boundaries. In addition to the altered neurites themselves, changes in specializations originating from proximal dendrites can often be found (Fig. 3F). These may originate from the recently discovered spiny stellate NOS-containing neurons (27) but differ from normal spines in that they have very long and thin stalks connecting the dendritic shaft with the terminal dilatation of the process (arrows in Fig. 3F). Similarly altered dendritic spines have been observed in patients with chromosomal aberrations and mental retardation (31).

In addition to the far more abundant neurite-like dystrophic processes, numerous masses of NADPH diaphorase-positive material within the size range of normal NOSN occur in patients with MND and dementia. These masses are often associated with neurite-like dystrophic processes that surround and often arise from them, indicating that they are most probably dystrophic NOSN perikarya that may eventually die (Fig. 3C–E, hollow arrows).

Dystrophic changes in NOSN were also seen in patients with Alzheimer’s disease (Fig. 4) and were consistent with previous reports indicating that NADPH diaphorase- and neuroactive-peptide-containing neurons (in which NOS is co-localized) are variably involved (32–37) or spared (38, 39) in this condition. In general, however, dystrophic changes in NOSN consistent with previous reports were less abundant and harder to find in patients with Alzheimer’s disease and thus appear less widespread than in MND, although they are qualitatively similar (Fig. 4). Neuropeptide Y-containing dystrophic neurites (that presumably co-localize NOS) have been reported in dementia with cortical Lewy bodies (19). These processes are quite numerous and large, indicating a probable parallel with similar changes in MND + D, addressed more extensively in the Discussion section.

Virtually no such NADPH diaphorase-positive dystrophic processes were observed in our patients with corticobasal degeneration, dementia with nonspecific changes, and progressive supranuclear palsy. It remains to be determined, however, if such changes do occur in series with larger numbers of similarly affected patients.

Selective Involvement and Sparing of NOSN Subtypes

While the dystrophic elements described above were situated in all cortical areas and layers examined, they did not affect all types of NOSN indiscriminately. A recent study revealed that there are multiple cytologically distinct types of NOSN (Fig. 1), with relatively selective areal and laminar distributions (27). In the present material, virtually all NOSN identified as abnormal correspond to the most abundant variety of NOSN: smooth stellate cells (Fig. 1C). We have no conclusive evidence that sparsely spiny cells are also affected (work in progress), but the putative dendritic spine alterations (Fig. 3F) probably occur predominantly in this cell type. By contrast, we have not yet seen a dystrophic NOS-positive local circuit neuron in layer I or a dystrophic granule cell in layer II. In fact, the latter appear normal so far in all cases examined (Fig. 5). Some caution is necessary to avoid prematurely ruling out alterations in these latter neurons, however, because they may be less numerous than degenerating smooth stellate cells, because they may be removed faster after developing dystrophic changes and dying, and because the size of the processes of granule cells is at or near the limit of resolution of the light microscope.

Fig. 3. Photomicrographs of NADPH diaphorase-positive dystrophic neurites (panel A–E, arrowheads), perikarya (panels C–E, hollow arrows), altered dendritic spines (panel F, straight arrows), and a probable astrocyte (panel G, curved arrow). Note the end feet of the astrocyte apposed to a neighboring blood vessel wall (arrowheads) and the smooth stellate cell nearby, for comparison with the abnormal cytological features in the preceding panels. From case A.
or NADPH diaphorase activity in the normal human brain (27); and (iii) we found a very small number of elements bearing the typical features of normal astrocytes (i.e. no axons, perikarya smaller than large pyramidal neurons but often larger than small local circuit neurons, arachnoidiform processes devoid of dendritic spines, and end feet apposed to blood vessels) in the white matter of patients with MND (Fig. 3G). Thus, while the latter occasional finding may reflect an abnormality indicating induction of NOS activity in a few astrocytes (40), this observation supports the probability that dystrophic astrocytes would have been easily recognized if they were present in substantial numbers and the putative identification of virtually all NOS-positive dystrophic elements as neuronal in origin. This is supported further by our inability to find dystrophic processes and massive or widespread astrogliosis in Holzer and glial fibrillary acidic protein preparations, which have nevertheless been shown to reveal such gliosis in some patients with MND (41, 42). No microglia-like, oligodendrocyte-like or macrophage-like NOS-positive elements have been identified in our material.

**DISCUSSION**

The present observations provide strong indications that NO synthase may be involved in the pathophysiology of cognitive impairment associated with MND. Although the precise biological substrate of non-Alzheimer and non-infarct dementia in MND remains to be elucidated, it is possible that the alterations we found in NO synthase may be responsible, at least in part if not primarily, for the non-motor manifestations of MND, and especially for the cognitive deterioration. In fact, the changes in NO synthase observed may induce cognitive impairment entirely by themselves, or perhaps in concert with additional transneuronal neurodegenerative changes consequent to the death and removal of NO synthase from the cortical circuitry.

This hypothesis must be tempered by the fact that the apparent absence of involvement of other elements within the cortical circuitry rests primarily on inherently inconclusive negative evidence and on the unproven assumption that dementia in MND is due to a single cause. It is also evident that our findings must be corroborated in a larger series of patients with MND, to verify their apparent correlation with dementia and to determine their place in the course of MND.

It may be especially significant that virtually all the neurons that appear affected belong to the smooth stellate type, and perhaps also to the recently discovered class of sparsely spiny NOS (27). This is in contrast to other smooth stellate cells, such as the local circuit neurons of layer I and the granule cells of layer II, both of which appear unaffected (Fig. 5A, B) and which occur in the immediate vicinity and surround clearly affected neurons. While it is possible that seemingly spared smooth stellate

**Possible Involvement of Glial Cells**

The purely cytological criteria used to identify the above degenerating elements as neurons containing NO synthase may present potential problems in ruling out a glial contribution to these altered profiles. We feel, however, that it is extremely unlikely that the dystrophic elements are glial in origin. In fact, at least 3 factors mitigate against this possibility: (i) virtually no degenerating elements resembling glia were encountered; (ii) normal glial cells rarely, if ever, exhibit constitutive NOS immunoreactivity

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**Fig. 4.** Photomicrographs of NADPH diaphorase-positive dystrophic neurites in layers II/III of a patient with Alzheimer's disease (case R, Table 1). These lesions can be subtler (e.g. panel A) and are harder to find in Alzheimer's disease than in MND with dementia but are qualitatively indistinguishable among patients with these two conditions.
NOSN would degenerate also, were the patients to survive MND long enough, the probability that there are at least several functionally different subtypes of neurons within this general category implies that these different subtypes may be selectively vulnerable or resistant to the disease process. This also suggests that, whatever the insult that affects smooth stellate neurons, it does not select merely for the NOS phenotype, but is at least relatively specific to certain NOS-containing cell types and not to others. This is consistent with the prediction that different types of NOSN may subserve different functional roles and that these presumed functionally as well as cytologically different sets of NOSN may be differentially susceptible to neurodegenerative conditions (27).

The present observations also open the possibility that pharmacological interventions aimed at replacing NO and/or co-localized transmitters/modulators (such as neuropeptide Y, somatostatin and tachykinins) produced by the NOSN that degenerate may be effective to palliate cognitive impairment in MND not due to other concomitant neurodegenerative disorders (e.g. Alzheimer’s disease, spongiform encephalopathy, which have been shown to occasionally co-exist with MND).

A Possible Mechanism Underlying the Dystrophic Changes in NOSN

The precise mechanisms responsible for the presence of dystrophic NOSN in ALS with dementia are not known, but hopefully will become susceptible of experimental approach shortly. One exciting possibility for future experimental exploration is based on the recent discovery of many different mutations in the gene for superoxide dismutase (SOD). These mutations are associated with a range of changes in the levels of SOD activity, and with an autosomal dominant pattern of familial ALS that accounts for a subset of all kindreds afflicted by this form of the disease (43–50). Although other families afflicted by ALS appear to have mutations that do not affect the SOD gene and the bulk of the sporadic cases of ALS do not seem to arise from defects in the SOD gene, the exciting discovery of Rosen et al (43) strongly indicates that a variety of deficiencies at a number of stages in the handling of reactive oxygen species may be the cause of the motor neuron diseases. Thus, a variety of both genetic and acquired defects in the processing of free radicals may converge into a unique disease phenotype with a range of clinical and histopathological manifestations. Interestingly, at least nine of the SOD mutations discovered so far do not affect the active site of the enzyme but a variety of sites in the side chains that may push open the protein loops that form the superoxide-binding pocket. This tertiary conformational change has been postulated to expose more copper in the SOD molecule, making it available for reaction with peroxynitrite (51). It is therefore reasonable to expect a certain amount of reduction in SOD activity in patients carrying only one allele of the familial SOD mutations, doubling the concentration of superoxide. Superoxide is known to react with NO at a rate of $6.7 \times 10^9$ M$^{-1}$ s$^{-1}$, which is about three times faster than with native SOD, to form the powerful oxidant peroxynitrite (ONOO$^-$) (52). Peroxynitrite then reacts with SOD to form a nitronium-like intermediate capable of nitrating tyrosine residues in multiple proteins, including SOD and NOS. This nitration process probably affects the function of numerous proteins, and this likely reduces superoxide scavenging further by a conformationally altered SOD. It is less clear what additional effects, besides the reduction in SOD activity, may result from another set of mutations that affect directly the catalytic site of SOD.
This hypothetical process may be enhanced in NOSN and in their vicinity, and may well account for the dystrophic changes observed in these neurons. This is predicated on the assumption that NOSN ordinarily have mechanisms to protect them from their ability to generate the highly reactive NO species, and that these mechanisms are somehow overwhelmed or ineffective in MND, due either to genetic or environmental factors. It remains to be determined precisely why dystrophic changes induced in NOSN by the above hypothetical mechanism appear to occur exclusively in patients with MND and non-Alzheimer dementia, as compared to our patients with MND but without dementia which did not exhibit similar pathology.

The Emerging Concept of Local Circuit Neuron Disorders

We find it intriguing that all NOSN in the mammalian neocortex belong to the category of local circuit neurons (LCN), i.e. nonpyramidal neurons "...whose axonal and dendritic processes are entirely confined within a given structure. For example, within a specific nucleus, ganglion or cortical area..." (53). These comprise an important group of neurons that are in most cases the principal components of local neuronal circuits, where neuronal operations variably referred to as the "computations" and "transformations" of information processing take place. The functional role of LCN in this setting may be to integrate information at the local level, rather than to relay messages to a different structure. The Spanish neurohistologist Santiago Ramón y Cajal recognized by the last turn of the century the importance of these neurons (54), which are variably designated by the (not entirely synonymous) terms: "Golgi type II neurons," "interneurons," "stellate cells," "granule cells," "modulators," "modifiers," "multipolar" neurons," and "intranuclear neurons" (53). In fact, it is the late genesis of LCN during ontogeny, compared to the early genesis of projection neurons, and the apparent capacity they have to change their morphological, physiological and biochemical phenotypes in response to experience and, presumably, various pathological processes that has been regarded as an important biological substrate for brain adaptation in response to a constantly changing environment (53).

The study of LCN had until recently been constrained to laborious analyses using the Golgi method and electron microscopy. This approach is especially cumbersome for the analysis of human neuropathologic conditions. Fortunately, this situation has changed in recent years with the recognition that LCN contain virtually exclusively certain molecules that are easy to demonstrate with modern histo- and immunocytochemical methods. These methods are capable of detecting substances such as neuropeptide Y, tachykinins, δ-aminobutyric acid (GABA) and calcium-binding proteins such as parvalbumin and calbindin, in addition to NOS. These molecules are not only markers of LCN but also make reliable cytochemical labeling of their entire cell body and processes possible, allowing in the identification of morphologically, physiologically and molecularly unique subtypes of LCN (56). A new era is thus dawning in the investigation of LCN, which may reveal their precise functional role.

The present findings open the possibility that relatively selective involvement of LCN underlies, at least in part, the cognitive impairment in MND. There is some evidence that one additional condition may result from a predominant involvement of LCN: dementia with cortical Lewy bodies (DCLB; variably known also as Lewy body disease, diffuse Lewy body disease and the Lewy body variant of Alzheimer’s disease). In this condition, intracellular neurofibrillar inclusions similar to the Lewy bodies of idiopathic Parkinson’s disease occur not only in large monaminergic pigmented nuclei of the brain stem but also in a number of other regions of the brain, including LCN of the cerebral cortex (18, 19). In an intriguing parallel with the present report, patients with DCLB exhibit large numbers of dystrophic neurites that contain neuropeptide Y (19). This is significant because this neuroactive peptide has been shown to co-localize with NOS in the same population of nonpyramidal LCN in the cortex (32, 36, 55), and they share a range of cyto logically distinct cell types common to nonhuman pri mates (56) and humans (27). As in MND + D, the precise basis for the dementia in DCLB remains unknown, but it would now appear reasonable to propose that—in both conditions—the cognitive defect is mediated in part by a pancellular degeneration of LCN that co-contain NOS and neuropeptide Y. This would result in diffuse alterations of local neuronal cortical circuits that are essential for normal cognitive operations. One of the most appealing aspects of this working hypothesis is that it would provide a unifying explanation for seemingly disparate conditions by invoking a lesion predominantly involving LCN that could even by sufficient, in and of itself, to cause progressive cognitive impairment in the absence of additional lesions undermining cortical circuitry.

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