Letters to the Editor

Dopaminergic Neurons Degenerate by Apoptosis in Parkinson’s Disease

Using in situ end labeling of DNA fragments, Banati et al. were unable to detect cells with DNA fragmentation in the substantia nigra of 10 patients with idiopathic Parkinson’s disease. From their study, they concluded that apoptosis is not a major feature in Parkinson’s disease. However, this conclusion should be taken with caution because three other studies indicate the presence of apoptotic neurons in the substantia nigra of patients with Parkinson’s disease. Indeed, using DNA end labeling, Mochizuki et al. found cells displaying DNA cleavage in the substantia nigra of four patients with Parkinson’s disease in contradiction with the findings of Banati et al. The difference between these two studies is difficult to explain but may be the result of differences in the sensitivity of the techniques used. Similarly, Anglade et al. found the typical features of apoptotic neurons in the substantia nigra of three patients with Parkinson’s disease using electron microscopy. In this study, the typical morphological characteristics of apoptosis were observed, including condensed chromatin in the nucleus of neurons containing neuromelanin, with intact cytoplasmic membranes. Furthermore, the presence of apoptotic bodies engulfed in glial cells, an event that occurs at the latest stage of the apoptotic process, indicated that the apoptotic neurons were not simply detected as a consequence of postmortem cell degradation. The ultrastructural approach allowed the authors to exclude the possibility that DNA cleavage solely occurred as a consequence of nonspecific DNA fragmentation. Cells displaying the features of autophagic degeneration were also seen in this study, indicating that apoptosis is not the only mechanism by which dopaminergic neurons degenerate. More recently, using fluorescent probes specific for both DNA cleavage and chromatin clumping, Tatton and coworkers were able to confirm, at the light microscope level, the positive staining of melanized neurons in the substantia nigra pars compacta. Staining with both techniques in individual neurons thus unambiguously confirms the presence of apoptosis. Taken together, these studies indicate that apoptosis can occur in Parkinson’s disease. This concept is further supported by the fact that a proapoptotic transduction pathway, depending on tumor necrosis factor α (TNFα) receptor activation, is probably induced in the substantia nigra of patients with Parkinson’s disease. This latter study is in agreement with the presence of activated microglial cells reported in the study of Banati and others and glial cells expressing TNFα.

Another issue raised in the paper by Banati et al. is that in the parkinsonian brain, a limited number of astroglial cells could be detected and their number was not increased compared with control brains. This is also in contrast with a previously published quantitative study in which the density of astroglial cells has been shown to be increased by 1.7-fold in the substantia nigra of patients with Parkinson’s disease. This difference again may be the result of a lack of sensitivity of Banati’s analysis, or the fact that their study was only qualitative and that a 1.7-fold increase is difficult to evaluate qualitatively. The role played by these cells is not yet known, but because these cells can secrete neurotrophic factors and contain free radical scavenging enzymes, it is likely that at least some of them have a neuroprotective role. From a more general point of view, the presence of both astroglial and microglial cells in the parkinsonian substantia nigra highlight the fact that glial cells may participate in the pathophysiology of neurodegenerative disorders.

In conclusion, the presence of apoptotic cells identified independently by three groups of investigators using different techniques seriously questions the negative findings of Banati and coworkers.

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What Does Apoptosis Have To Do With Parkinson’s Disease?

Dr. Hirsch and colleagues’ letter propounds the view that nigral neurons in Parkinson’s disease (PD) die mainly as the consequence of one specific cellular mechanism, “apoptosis.” This is at odds with several recently published reports1–3 in which we and others were unable to confirm neuronal apoptosis as a major feature of PD in the substantia nigra of more than 30 well-studied cases.

The three studies4–6 quoted by Hirsch and colleagues are generally limited first by the small numbers of patients studied, and second by the relative lack of suitable control material. As others have already remarked, the study of three PD patients by Anglade et al.7 has, in fact, failed to demonstrate the characteristic features of apoptosis. Further, the absence of any data from age-matched normal brains precludes judgment about whether their observations are significant in terms of a nigral pathology specific for PD. The studies by Mochizuki et al.8,9 report apoptotic neurons in the nigra of four of seven patients with PD using in situ DNA end labeling. This technique is prone to false-positive detection of apoptosis, particularly if labeled cells without clearly apoptotic morphology, whether they are glial or neuronal, are included.10–13 In a preliminary study combining different fluorescent DNA-labeling techniques with subsequent image processing to improve resolution, Tatton et al.6 report on “apoptotic” neurons in the nigra of a single patient with PD. This preliminary study awaits confirmation, but even if reproduced, the new criteria used are not part of the classic definition of apoptosis.14 In addition, some morphological features seen by light microscopy after fluorescent and non-fluorescent DNA labeling, such as apparent chromatin margination, may, at the electron microscopic level, be identified as perinuclear staining uncharacteristic of classic apoptosis.15,16

Contrary to these three reports of “apoptosis” found in a small and heterogeneous set of patients are the findings of a number of recent studies in which an increased rate of neuronal apoptosis was not found.1,4 In an extended investigation of 22 brains from patients with PD, Kösel et al.,2 using in situ DNA end labeling, failed to detect apoptotic neurons in the nigra. Instead, they found end labeling in glial cells and some reticulin staining of neurons but not a single nerve cell showing classic features of apoptosis.14 Similarly, in our own study of 10 PD brains and three controls,1 DNA end labeling revealed the infrequent presence of labeled apoptotic cells throughout the entire brain (including white matter) in normal and diseased brains alike. In contrast, although no problem with the sensitivity of the technique to detect truly apoptotic nuclei was encountered, apoptotic neurons in the nigra were not found.

There are a number of other reasons why it is difficult to endorse Dr. Hirsch and colleagues’ view that apoptosis in the parkinsonian nigra is the pathologically relevant cause of disease: the frequency with which supposedly apoptotic cells are found in PD (up to 4.8%)8 appears essentially incompatible with the protracted time course of a chronic neurodegenerative disorder as exemplified in the case of Alzheimer’s disease.13 The evidence that testifies to the presence of apoptosis-specific proteins is equally conflicting.17–19 Undoubtedly, the present controversy reflects, in part, the weakness of the methodology (in our view with a tendency of over-rather than underreporting) and better markers for the various cell death-associated processes are clearly needed. Whereas, importantly, there appears to be consensus that neuronal changes in PD involve the nucleus and its DNA, the interpretation of these findings differs widely. It may, therefore, be appropriate to discuss the problem of DNA fragmentation and nerve cell death also in relation to other neurodegenerative disorders. In more acute neurodegenerative disease, such as amyotrophic lateral sclerosis (ALS), it may be difficult to support the claims that apoptosis is the primary cause of disease rather than a secondary event, possibly related to the severity of the clinical condition. Kihira et al.,16 based on the study of 17 patients with ALS, did not find significantly increased apoptosis above an incidental occurrence. The authors, however, measured significant non-apoptotic nuclear changes in ALS neurons. Consequently, they suggested that while apoptosis may be related to terminal events, the form of cell death characteristic of the disease proper is different from classic apoptosis. Similarly, apoptosis now seems unlikely as a major mode of cell death in Alzheimer’s disease.13

Yet the term “apoptosis” also appears to be used somewhat indiscriminately in the literature. We may remind ourselves why the argument over whether cell death occurs through apoptosis or other forms of cell death is generating such interest. After all, does it really matter how cells die? Indeed, it does. There is no dissent that the term apoptosis implies a specific, active program of cell death as a primary cause of cell loss (albeit it may also be triggered by certain external stimuli) which in turn intimates the possibility of an equally specific “anti-apoptotic” as opposed to a more general “neuroprotective” therapy. Widening the original meaning of “apoptosis” to include various disparate “pre-apoptotic” states would liken it to the coroner’s notorious blanket diagnosis of “heart failure” which is as all-encompassing as it is uninformative. Recently, the term aposklesis (“withering”) has been proposed to distinguish the non-apoptotic and apparently slow death of neurons in neurodegenerative diseases such as Parkinson’s from the fast and physiological, morphologically well-defined apoptotic type of cell death which occurs during development.20

We would, therefore, like to re-emphasize that in long-standing PD (duration 7–41 years and age of onset 30–73 years), neuronal apoptosis does not appear to represent a major feature of nigral pathology.

Lastly, Dr. Hirsch’s letter mentions the hypothetical contribution of glial cells to the etiology of PD. We agree that glial cells may play an important role in the pathogenesis of neuronal degeneration. The microglial marker used in our study is only expressed in activated microglia and is absent in the normal paraffine-fixed brain parenchyma, thus allowing a qualitative assessment of absence or presence of a disease process in general. This is not the case with the marker for reactive astrocytes (that is, glial acidic fibrillary protein [GFAP]) that can also be expressed in areas without pathology. However, if, as in
our study, massive astrogial scarring is not found, a meaningful quantitation of moderate changes, concomitant to obvious changes in the cytoarchitecture and volume of the parkinsonian nigra compared with controls, is not trivial and would require appropriate stereologic controls. However, our study has not attempted to furnish any data pertaining to the relative importance of the different glial subpopulations. Microglial staining served primarily as an indicator of ongoing disease processes.

It is noteworthy that particularly the concept of microglia as inflammatory cells contributing to neuronal cell death, rather than being its primary cause, has undergone significant sophistication. It is becoming increasingly clear that microglia/brain macrophages potentially secrete factors promoting regeneration and cell survival. Hence, if these cells are thought of as therapeutic targets, their regulation rather than mere suppression may have to be the goal. Sadly enough, most of our knowledge is based on in vitro experiments and confirmation in vivo is largely unavailable. Here, too, new methods are needed to study cell biologic processes in real life rather than having to extrapolate from postmortem material.

As made explicit in the discussion section of our studies, our findings do not rule out the principal possibility of apoptotic neurons occurring in the PD nigra. For the time being, however, the claim that apoptosis represents a major feature of chronic PD, as opposed to an incidental finding that may occur also in age-matched normal brains, clearly lacks support.

If the specific process of apoptosis is not viewed as simply a final common pathway, it seems plausible that the heterogeneity of disease causes leading to familial or sporadic forms of PD is reflected in a heterogeneity of cell death mechanisms. The simple dichotomy necrosis versus apoptosis may no longer suffice.

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Clinical Assessment of Olfactory Dysfunction in Parkinson’s Disease

We read with interest the article “Clinical Assessment of Olfactory Dysfunction in Parkinson’s Disease” by Potagas et
al. in which they conclude that patients with Parkinson’s disease (PD) have significantly poorer odor identification compared with healthy control subjects, yet intact odor discrimination, indicating a problem with the long-term memory of odors. They suggest that compared with healthy subjects, long-term olfactory memory is affected in PD.

We have also investigated olfactory function in 20 patients with clinically probable PD and 20 healthy age-matched subjects using a simple, five-smell identification kit comprised of peppermint, asfoetida, coffee, cloves, and turpentine. We similarly found that odor identification by PD patients was significantly impaired compared with the control group (15 of 20 patients were unable to identify any of the five smells compared with one of 20 healthy subjects). We also found that 12 of 15 PD patients who were unable to identify any of the five smells could still detect “a smell,” again suggesting that in PD odor identification difficulty may be the result of odor memory impairment. However, unlike the reported study, we also found evidence of impaired odor discrimination in our patient group. Our group of patients who were unable to identify any of the smells were also unable to discriminate odors in the five-smell sequence after a delay period even when given verbal cues. This implies that central long-term odor memory and short-term memory or peripheral capture of olfactory information are both impaired in PD compared with age-matched healthy subjects.

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Drug-Induced Dystonia and Dysphonia in Parkinson’s Disease

We read with interest Warren’s report on drug-induced dystonia and dysphonia in a case of idiopathic Parkinson’s disease. It is well known that metoclopramide can cause acute dystonia, including upper airway obstruction, even in patients without extrapyramidal disorders. Warren et al. gave their patient a small dose of levodopa. However, we recommend intravenous injection of 5 mg biperiden in patients who are suspected of having metoclopramide-induced dystonia because biperiden will work rapidly and ameliorate dystonia induced by metoclopramide. On the other hand, we are interested in the dysphonia manifested by this patient. We previously encountered a patient with idiopathic Parkinson’s disease who developed dysphonia and orolingual dykinesia induced by trihexphenidyl. The vocal cords had normal movement on phonation and no abnormal involuntary movement at rest. The false vocal cords excessively adducted only on phonation, and it was considered dysphonia plicae ventricularis. After discontinuing trihexphenidyl and administration of sulpiride, the dysphonia and orolingual dykinesia disappeared but parkinsonism worsened. Rechallenge with trihexphenidyl again provoked the same abnormal phonation and orolingual dykinesia. We speculated that dysphonia in our patient resulted from overactivity in the balance between the dopaminergic and cholinergic systems in controlling phonation. It is important to bear in mind that abnormal extrapyramidal symptoms of the upper airway in a patient with idiopathic Parkinson’s disease could be induced not only by anti-dopaminergic drugs but also by anticholinergic drugs.

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