Abnormal expression of two microtubule-associated proteins (MAP2 and MAP5) in specific subfields of the hippocampal formation in schizophrenia

(central nervous system/subiculum/entorhinal cortex)

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ABSTRACT A variety of cytoarchitectural disturbances have been described in limbic regions in postmortem studies of schizophrenia, many of which suggest a developmental disturbance of normal neuronal geometry. This geometry is established and maintained by elements of the neuronal cytoskeleton. Immunohistochemistry with a panel of 15 monoclonal antibodies was used to monitor the presence of neuronal cytoskeletal proteins in the hippocampal formations of six patients with schizophrenia, six normal controls, and six with neurodegenerative disorders. In five of the six patients with schizophrenia, prominent and specific alterations were found in the distribution of two microtubule-associated proteins, MAP2 and MAP5, which were anatomically selective for the subiculum and entorhinal cortex. In contrast, the immunoreactivity of other cytoskeletal proteins (i.e., tau, tubulins, and selected neurofilament protein phosphoisoforms) was similar for all subjects. Defects in the expression of MAP2 and MAP5, two proteins that contribute to the establishment and maintenance of neuronal polarity, could underlie some of the cytoarchitectural abnormalities described in schizophrenia and impair signal transduction in the affected dendrites. The subiculum and entorhinal cortex interconnect the hippocampal formation with widespread cortices and subcortical nuclei and play important roles in higher cognitive functions. Hence, pathologic lesions that distort the polarized geometry of neurons could play a role in the emergence of aberrant behavior in schizophrenia.

Although a variety of cytoarchitectural abnormalities in limbic structures of patients with schizophrenia have been described, the specificity of these alterations for schizophrenia and their etiology remain unknown. Most pathologic studies of schizophrenia have used conventional chemical stains, which lack molecular specificity because the stains exhibit an affinity for a number of cellular components. Nevertheless, these methods have revealed a variety of abnormalities in neuronal polarity (1-3) as well as in the quantity (2, 5-9), laminar positions (4, 8), or spatial arrangement (10, 11) of selected neurons of limbic areas. Many of the findings have been ascribed to abnormal cortical development, rather than to a neurodegenerative process. Accordingly, the failure to establish a highly ordered cortical cytoarchitecture during development would most likely coincide with disturbed connectivity between the hippocampus and related limbic and non-limbic regions. The reduced fidelity of neuronal circuits may lead to behavioral disturbances.

The molecular basis of these various and subtle perturbations of limbic cortical cytoarchitecture is obscure. We hypothesized that some of the cytoarchitectural abnormalities described could result from a developmental defect in the establishment of the highly asymmetric three-dimensional geometry of neurons, which distinguishes them from all other mammalian cells. The structural and molecular basis of neuronal polarity is determined in part by a well-characterized family of neuronal cytoskeletal proteins, many of which are expressed exclusively in functionally and anatomically distinct domains (axons, dendrites) of neurons (12-17). These proteins could be the targets of neuropathological events that ultimately result in aberrant behavior. For these reasons, we used monoclonal antibodies (mAbs) to investigate the distribution of a key group of developmentally regulated neuronal cytoskeletal proteins in the hippocampal region of postmortem brain samples from patients with schizophrenia. Among the large group of proteins examined here, prominent abnormalities were noted in the immunoreactivity of only two microtubule-associated proteins (MAPs), MAP2 and MAP5 (alternatively known as MAP1B), and these abnormalities were largely confined to specific subfields of the hippocampal formation in patients with schizophrenia.

MATERIALS AND METHODS

The cases studied were listed with clinical and neuropathological findings in Table 1. Brains were obtained at autopsy from six chronically institutionalized psychiatric patients (five with schizophrenia, one with schizoaffective disorder, ages 68-86), six neurologically normal controls (ages 16-91), and six controls with a neurodegenerative disease (ages 58-81). Psychiatric diagnoses were made by applying DSM-III-R criteria (31) on chart review. All cases had complete autopsies and neuropathological examinations. The brains of five of the six patients with schizophrenia had no diagnostic neuropathologic lesions, whereas the sixth brain had a sufficient number of neocortical senile plaques to meet histopathologic criteria for AD. However, the hippocampal formation from this patient exhibited no AD pathology.

Blocks from at least four rostral-caudal levels of the hippocampus and parahippocampal gyrus from each brain were sectioned at a thickness of 6 μm and processed for immunohistochemistry using the peroxidase/anti-peroxidase method as described (18, 19). Approximately 30 different mAbs, specific for each of the major brain MAPs (MAP2, MAP5, tau) as well as α- and β-tubulin and diverse neurofilament (NF) protein phosphoisoforms, were used in preliminary studies. Fifteen mAbs were then selected for a detailed analysis of the polarized arrangement of cytoskeletal proteins in hippocampal neurons in the schizophrenic and normal controls.

Abbreviations: E.C., entorhinal cortex; mAb, monoclonal antibody; MAP, microtubule-associated protein; NF, neurofilament.

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control cases (Table 2; refs. 20–25). Sections adjacent to
those probed by immunohistochemistry were stained with
cresyl violet for standard cytoarchitectural assessment. Tis-
tue sections were blindly rated as normal or abnormal by two
of the authors and fully characterized.

RESULTS

Deficits in the normal pattern of immunolabeling of MAP2
and MAP5 in the hippocampal region were found in five of the
six psychiatric patients. No deficits were found for tau,
tubulins, or NF proteins.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Neuropsychiatric diagnosis</th>
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<th>Psychotropic treatment</th>
<th>MAP2</th>
<th>MAP5</th>
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<tbody>
<tr>
<td>1 (L)</td>
<td>86/M</td>
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<td>Neuroleptics</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2 (R)</td>
<td>68/M</td>
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<td>Normal</td>
<td>Neuroleptics, lithium</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 (L)</td>
<td>75/F</td>
<td>Schizophrenia; tardive dyskinesia</td>
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<td>Neuroleptics</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 (L)</td>
<td>76/F</td>
<td>Schizoaffective; tardive dyskinesia</td>
<td>Normal</td>
<td>Neuroleptics, lithium; ECT</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5 (L)</td>
<td>78/M</td>
<td>Schizophrenia</td>
<td>&quot;Plaque-only&quot; AD; few cortical Lewy bodies</td>
<td>Neuroleptics</td>
<td>-</td>
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The left (L) or right (R) hippocampus examined in each case is indicated in parentheses. The presence (+) or absence
(−) of MAP2 and/or MAP5 staining in neurons of subiculum (SUB) or entorhinal cortex (EC) is shown in the last three
columns (ND, not done). AD, Alzheimer disease; PD, Parkinson disease; DLBD, diffuse Lewy body disease; ECT,
electroconvulsive therapy.

*Diminished immunoreactivity only in superficial layers.
†AD case with severe neuron loss.

Table 1. Subject data

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Normal Pattern of MAP2 and MAP5 Immunoreactivity in the Hippocampal Region. Neurons in the subiculum of the
normal controls exhibited the most robust MAP2 and MAP5 immunoreactivity of any hippocampal subfield (Fig. 1 a and
b; Fig. 2 b and c). This was especially intense in the dendrites of vertically oriented pyramidal cells and the fusiform neu-
rons and their processes adjacent to the angular bundle. In the EC, moderate numbers of MAP2-immunoreactive
neurons were present in layers II, V, and VI, while occasional MAP2-positive neurons were seen in layers III and IV. The
immunoreactive neurons in layer II often appeared in clusters. In contrast with MAP2, MAP5 immunoreactivity in
neurons in the EC was weaker and MAP5-positive neurons were rare. Notably, except for two controls with very severe
AD pathology, the distribution and intensity of MAP2 and MAP5 immunoreactivity in the hippocampal region of the
neurodegenerative controls was indistinguishable from that of
the normal controls. Specifically, one of the severe AD
cases (case 15) showed diminished MAP2 labeling of the EC
and subiculum, while the other case (case 16) showed de-
creased MAP2 labeling only in the EC. Since these alter-
ations were accompanied by extensive neuron loss, gliosis,
and neurofibrillary pathology, the abnormal expression of
MAP2 and MAP5 was distinct from the abnormalities de-
scribed below in the patients with schizophrenia.

MAP2 and MAP5 Immunoreactivity Pattern in Schizophreni-
a. In contrast to the controls, there was a marked paucity of
somatodendritic MAP2 immunoreactivity that selectively
affected neurons in the subiculum in five of the six psychiatric
cases and the EC in four of the six. This diminution in MAP2
staining was so dramatic that the hippocampal sections of
these four schizophrenia cases could be distinguished from
the control sections by macroscopic inspection (compare Fig.
DISCUSSION

Although the sample size was relatively small, there were profound abnormalities in five of the six psychiatric patients with a lack of similar alterations in the neurologically normal subjects or in the neurodegenerative disease controls (except for two severe AD cases which had extensive neuron loss). Thus, the results may warrant the speculation that disturbances in the expression of MAP2 and MAP5 represent anatomically selective and highly specific molecular correlates of psychiatric dysfunction in at least a subset of patients with schizophrenia. Another consideration in interpreting our findings is that the diminished MAP2 and MAP5 immunoreactivity in the schizophrenic sample could have been a result of long-term psychotropic medications and other somatic treatments (e.g., electroconvulsive therapy). However, the absence of similar MAP2 and MAP5 abnormalities in a control patient who was chronically treated with thioridazine (for behavioral problems associated with diffuse Lewy body disease) or in another control who received electroconvulsive therapy (for depression) argues against this.

Taken together, these data, as summarized in Table 1, suggest that the marked reduction in MAP2 and MAP5 immunoreactivity in patients with schizophrenia does not reflect a generalized abnormality of the neuronal cytoskeleton. Rather, we have identified highly selective abnormalities of two cytoskeletal proteins that specifically affect particular populations of neurons in the hippocampal formation (i.e., those in subiculum and EC).

While it is unclear whether the abnormalities described here are mechanistically linked to the emergence of the full schizophrenic phenotype, it is plausible that they account for some of the cytoarchitectural alterations that have been observed in the hippocampal formation in schizophrenia. Both MAP2 and MAP5 promote the polymerization of tubulin in vitro (12, 13) and stabilize microtubules (14–16), which are thought to play an important role in the establishment and

Fig. 1. Low-power photographs demonstrate the macroscopically evident differences in MAP2 (labeled with M12) and MAP5 (labeled with anti-MAP5) immunoreactivity between hippocampus of a normal elderly control (case 7) (a and b) and hippocampus of a schizophrenic (case 3) (c and d). The control parahippocampal gyrus (a) shows especially robust MAP2 labeling in deep and superficial layers of the EC and in the subiculum (SUB), while the schizophrenic parahippocampal gyrus (c) shows a paucity of neuronal immunoreactivity that is most evident in the EC and subiculum. Note the selectively preserved neuronal and neuropil labeling in the deeper layers of the EC in this case. Strong MAP5 immunoreactivity is seen in the control subiculum (c), while the subiculum of the patient with schizophrenia is almost negative (d). DG, dentate gyrus. Sections were counterstained with hematoxylin. (×6.5.)

1 a and c). In two cases (cases 1 and 4) only rare immunoreactive neurons were present in all layers of EC, while in the other two (cases 3 and 5), the diminished MAP2 immunoreactivity was confined to the superficial layers of the EC. In another case (case 2), MAP2 immunoreactivity was normal in the EC but diminished in the subiculum. The reduction in subicular MAP2 staining was paralleled by a distinct and marked diminution of MAP5 immunoreactivity in subicular neurons in the same cases (Fig. 1 b and d).

In sharp contrast to the two AD cases which had extensive neuron loss along with reduced MAP2 and MAP5 immunoreactivity, the hippocampal formations of the patients with schizophrenia contained abundant neurons and did not show evidence of gliosis or neurofibrillary changes in preparations examined using conventional stains and mAbs (i.e., mAbs to glial fibrillary acidic protein, tau, and ubiquitin; data not shown). However, Nissl staining revealed subtle cytoarchitectural alterations. These included poorly formed clusters of layer II neurons in the EC and dysmorphic subicular neurons (compare Fig. 2 a and e). However, these findings were much less dramatic than the alterations in the expression of MAP2 (Fig. 2 b and f) and MAP5 (Fig. 2 c and g) in these regions.

Other Cytoskeletal Proteins. Immunohistochemistry with antibodies directed at other protein elements of the neuronal cytoskeleton revealed no differences between schizophrenic and control cases. For example, RMDO20, a mAb specific for the poorly phosphorylated mid-sized NF protein (which, like MAP2 and MAP5, is largely confined to the somatodendritic domain of neurons), labeled neurons in the hippocampal formation of control cases and all but one schizophrenic case in the same manner (compare Fig. 2 d and h). In this case (case 3), RMDO20 immunoreactivity appeared abnormally sparse in the superficial layers of the EC. Immunoreactivity for tau, α- and β-tubulin, and other NF protein phosphoisoforms in the hippocampal formation of the schizophrenia cases was indistinguishable from that in the controls (data not shown).
maintenance of neuronal polarity (17). In addition, MAPs are believed to mediate interactions of microtubules with each other and with other organelles (16). Hence, it is attractive to speculate that reduced expression or posttranslationally modified forms of MAP2 and MAP5 in schizophrenia could affect the equilibrium between polymerized microtubules and unpolymerized tubulins. If this affects the stability of dendrites or the ability of dendrites to maintain and remodel synaptic contacts, signal transduction across these specialized neuronal processes could be impaired.

Given the unique connectional neuroanatomy of the hippocampal formation and its role in higher cognitive functions (26, 27), faulty synaptic transmission across unstable, MAP2- and MAP5-deficient dendrites that extend from neurons in the EC and subiculum could have profound behavioral implications. The EC and subiculum have widespread connections with higher order multimodal and sensory-specific association cortices in all four lobes as well as with subcortical limbic structures, including the amygdala, thalamus, hypothalamus, basal forebrain (26, 27), and dopaminergic ventral tegmentum (28, 29). If impaired synaptic transmission is one of the consequences of diminished MAP2 and MAP5 expression in dendrites of neurons in the subiculum and EC, this might affect the fidelity of neuronal circuits or even serve to functionally disconnect the hippocampus from much of the brain. It may also alter limbic dopaminergic activity. Furthermore, if such disturbances occur early in brain development, the normal neural circuitry of the hippocampal region could be reorganized, as has been described in other neural systems (30). This process could fail to reconstitute normally functioning networks of interconnected neurons in schizophrenic patients.

Several mechanisms might account for the diminished immunoreactivity of MAP2 and MAP5 in selected regions of the brains of schizophrenics. There could be a developmental failure to induce the transcription of MAP2 and MAP5 genes in subiculum and the EC, a genetic mutation, or selective proteolytic cleavage of normally translated protein. Alternatively, there may be extreme posttranslational modification (e.g., by phosphorylation) that would make MAP2 and MAP5 unrecognizable to our antibodies. There are many steps in the transcription, translation, and metabolism of MAP2 and MAP5, and disruption of any of these steps could lead to decreased immunoreactivity. If reduced levels of hippocampal MAP2 and MAP5 are phenotypic markers of at least a subset of patients with schizophrenia, it will be important to establish the etiology of these abnormalities, the nature of their anatomically selective occurrence, and how they contribute to the behavioral manifestations of schizophrenia.

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