Original Article

Expression of the *N*-Methyl-D-Aspartate Receptor Subunit R1 in the Developing Human Hippocampus

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ABSTRACT

It has been suggested that the *N*-methyl-D-aspartate (NMDA) receptor family plays a key role in synaptic plasticity and synaptogenesis that is essential for memory, learning, differentiation, and development. Although gene expression of these receptors has been analyzed in the experimental animal brain and in some diseases of the adult or elderly human brain, it has not been studied in the developing human brain. Using in situ hybridization, we investigated the expression of the *NMDAR1* gene in the hippocampi of 16 human neonates who were between 22 and 40 weeks of gestation and had no evidence of critical episodes of brain insult at autopsy. Signals for *NMDAR1* were detected ubiquitously at all developmental stages. Dense hybridization signals were uniformly detected in the granular cells of the dentate gyrus in all specimens. Stronger signals were observed in the larger-type pyramidal cells in the CA2 and CA3 regions compared with the pattern seen in the CA1 region in the smaller-type pyramidal cells. These results suggest that the *NMDAR1* gene is expressed at 22 weeks and possibly occurs earlier in neuronal cell bodies of the dentate gyrus and all CA fields of Ammon's horn and that the *NMDAR* plays an important role in constructing neuronal networks in developing human brains. (*J Child Neurol* 2006;21:236–239; DOI 10.2310/7010.2006.00060).

Based on pharmacologic analyses using a highly specific ligand, D-2-amino-5-phosphono-valerate, glutamate receptor channels are classified into *N*-methyl-D-aspartate (NMDA) channels and non-NMDA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] type, kainate type) channels. NMDA receptors have two types of subunits, NMDAR1 and NMDAR2.[1,2] Analyses of NMDA receptor expression during the development of the mouse and rat brain revealed that distribution of the NMDAR1 and NMDAR2 subunits (NR2A, NR2B, NR2C, NR2D) varied greatly depending on the stage of development.[3–5] These studies revealed that the NMDAR1 subunit was expressed stably throughout the brain in the prenatal and postnatal periods, whereas each of the four subunits of NMDAR2 differed in the timing and location of expression and channel properties. Later studies showed that the NMDAR1 subunit combines with various NMDAR2 subunits to form polymers, which function as NMDA receptors. However, no studies have reported NMDA receptor gene expression during the development of the temporal hippocampal region in humans. To explore the functional mechanisms of NMDA receptors in the developing human brain, we analyzed the expression of the *NMDAR1* gene in the developing neonatal hippocampus using in situ hybridization.

MATERIALS AND METHODS

Brain specimens from 16 premature and mature neonates at 22 to 40 weeks of gestation without a history of neurologic abnormalities were selected. Specimens were used only when informed consent was obtained for the purpose of pathologic studies and further scientific analyses. All brain specimens were fixed with 10% formalin, embedded in paraffin, and sectioned at 5 μm thicknesses. They were stained with hematoxylin and eosin and Luxol fast blue. Histologic findings were judged as normal by H.Y., J.H., and Y.N. Complementary DNA of human *NMDAR1* (Genebank accession no. L05666) was generously provided by Dr. Montal of the University of California at San Diego. A 294 bp fragment of human *NMDAR1* complementary DNA, sequence 2634 to 2928, was isolated by digestion of the complementary DNA with ApaI and subcloned into pZEM11z. Digoxigenin-labeled sense and antisense riboprobes were synthesized by in vitro transcription with SP6 and T7 promoter ribonucleic acid (RNA) polymerases, respectively. After prehybridization with 50% deionized formamide for 1 hour, hybridization was

Received March 12, 2004. Received revised August 5, 2004. Accepted for publication August 11, 2004.

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This study was supported by a grant from the Ministry of Health, Labor and Welfare in Japan.

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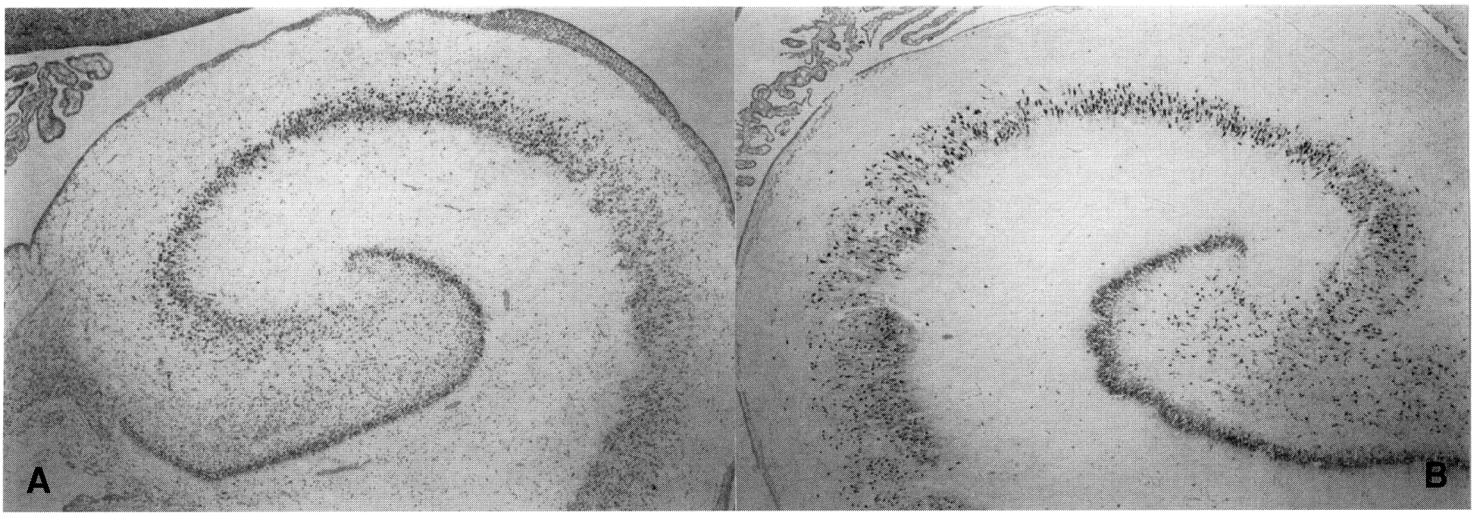

Figure 1. Histopathologic sections of Ammon's horn of the human temporal hippocampus and dentate gyrus (original magnification, ×40). *A,* In situ hybridization findings at 27 weeks of gestation. *B,* In situ hybridization findings at 40 weeks of gestation.

carried out overnight at 43°C with a 500 ng/mL of digoxigenin-labeled human NMDAR1 riboprobe in a hybridization buffer containing 2× saline-sodium citrate, 50% deionized formamide, 10% dextran sulfate, 1× Denhardt's solution, and 10 mM dithiothreitol. After hybridization and washing, the sections were incubated with an antidigoxigenin antibody that was conjugated with alkaline phosphatase and diluted to 1:500 at room temperature for 1 hour using a DIG Nucleic Acid Detection Kit (Boehringer Mannheim, Mannheim, Germany). Nitroblue tetrazolium chloride was used as a chromogen to visualize the hybridization signal.

RESULTS

In all hippocampal sections of neonates between 22 and 40 weeks' gestation, dense hybridization signals for NMDAR1 messenger RNA were detected uniformly and ubiquitously in the granule cells of the dentate gyrus and the pyramidal cells in Ammon's horn of the hippocampus (Figure 1). However, hybridized signal densities were different depending on the type of pyramidal cell. Weakly positive expression of *NMDAR1* messenger RNA transcripts was observed in the smaller-type pyramidal cells in the CA1 region of the human hippocampus (Figure 2, A and B). In contrast, the larger-type pyramidal cells in the CA2 and CA3 regions were strongly positive (Figure 2, C and D). The *NMDAR1* sense riboprobe always yielded a negative signal.

DISCUSSION

Some neurotransmitters and receptors change their function in the central nervous system according to the brain maturation. AMPA receptors are the main excitatory receptors in the mature brain but function incompletely in immature neurons.[6] γ-Aminobutyric acid (GABA) is regarded as the main inhibitory transmitter in the mature brain but it is the main excitatory transmitter in the fetal period.[6] As for NMDA receptors, studies have reported the expression of the *NMDAR1* gene in experimental animals,[3–5,7,8] young human adults,[9] and elderly patients with various diseases.[10–15] Because there have been no reports on the expression of NMDA receptors in the neonatal human brain, we explored gene expression in the neonatal human brain to understand the importance of NMDA receptor expression in the immature brain. Using in situ hybridization, we investigated the expression of *NMDAR1* messenger RNA in the temporal hippocampus of the fetal brain. We found that *NMDAR1* messenger RNA was expressed stably and uniformly in the human temporal hippocampus, with no gestation-dependent variation in expression pattern. In addition, this expression pattern agreed with previously reported in situ hybridization and immunohistologic findings in the brains of developing experimental animals.[3,4,7] In this study, we found that the signals in the larger type of pyramidal cells in the CA2 and CA3 regions were stronger than those seen in the CA1 region. These patterns of *NMDAR1* gene expression in the hippocampus have also been found in the adult brain.[16] We could not know precisely why the expression of this gene is different in each region, but the evidence suggested the importance of early establishment of *NMDAR1* gene expression for wiring of the neuronal network in the early period.

Analyses of gene expression in the developing brain of mice and rats have found that the *NMDAR1* gene is stably expressed ubiquitously in the prenatal period.[3,7] On the other hand, the four types of *NMDAR2* genes have been reported to change the distribution patterns of expression dynamically over time, from the early developmental stage to the maturation period.[3,4,7] Based on these reports, it has been suggested in animal experiments investigating brain development that the expression of the NMDAR1 subunit is essential for individual *NMDAR2* genes to change distribution patterns of expression.[3,17–20] Several studies have reported that the *NMDAR1* gene product works by forming a polymer (heteromeric channel) with each *NMDAR2* gene product, and the *NMDAR1* gene is the most important and essential subunit for the expression of NMDA receptor channels.[3,16,21] Thus, the NMDAR1 subunit is essential for NMDA receptor channels to function. NMDA receptors are localized in the postsynaptic membranes of neurons and have two properties: membrane potential–dependent control and high Ca^{2+} permeability. It is currently suggested that NMDA receptor channels play an important role in synaptic plasticity in the hippocampus, synaptogenesis that is essential for memory, learning, and differentiation; during neuronal development and for neuronal cell necrosis under various pathologic conditions.[22–25]

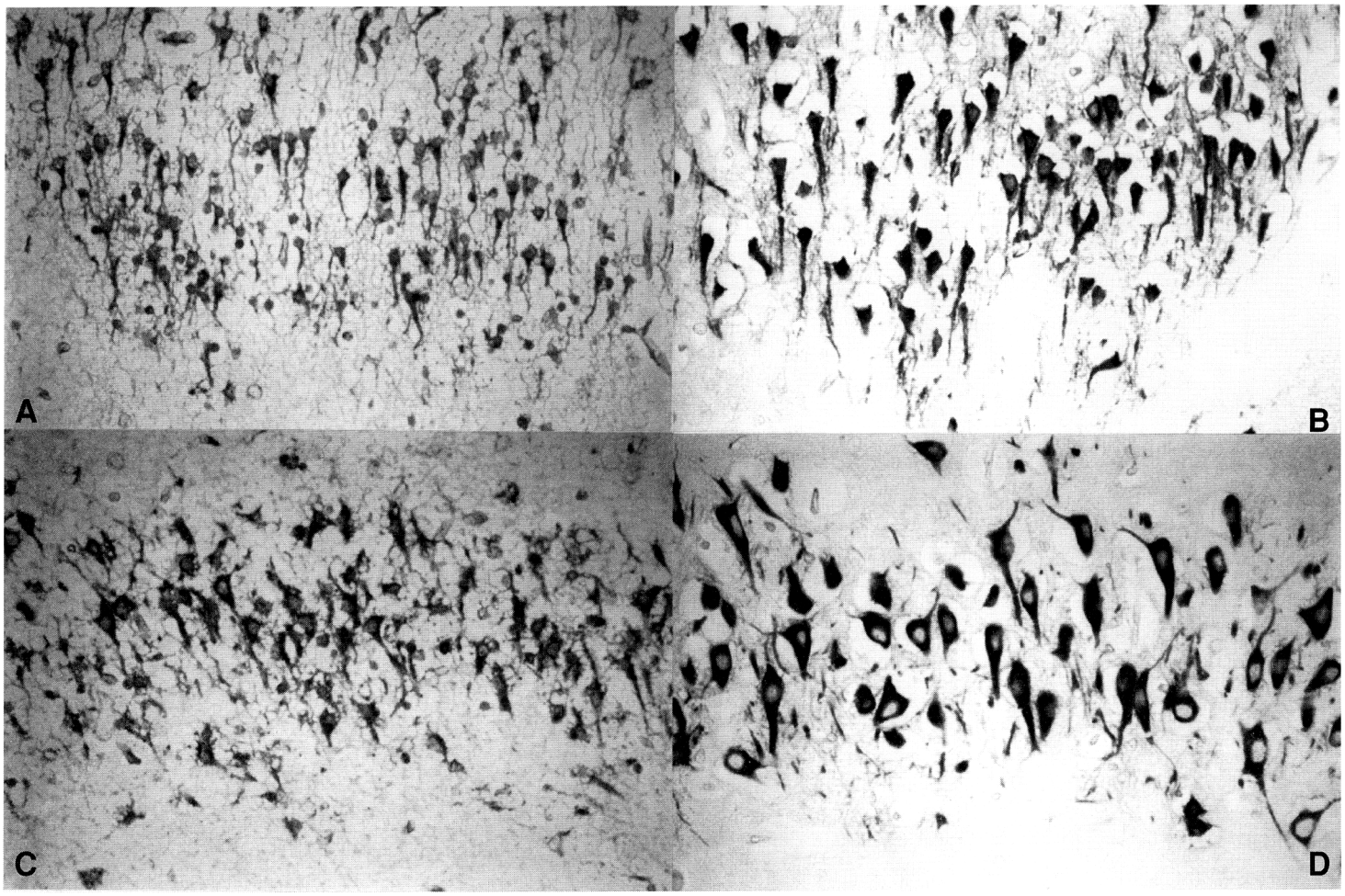

Figure 2. The expression of *NMDAR1* messenger ribonucleic acid (RNA) as demonstrated by in situ hybridization in the pyramidal cell layer of the human hippocampal CA1 and CA2/CA3 regions (original magnification, ×400). *A* and *B,* In situ hybridization findings at 27 weeks (*A*) and 40 weeks (*B*) of gestation in smaller-type pyramidal cells in the region of CA1, which exhibited weakly positive hybridization signals of *NMDAR1* messenger RNA. *C* and *D,* In situ hybridization findings at 27 weeks (*C*) and 40 weeks (*D*) of gestation in larger-type pyramidal cells in the region of CA2/CA3, which revealed strongly positive expression of *NMDAR1* messenger RNA hybridization signals.

In summary, our analysis of *NMDAR1* gene expression during the development of the human temporal hippocampal region showed that the *NMDAR1* gene was already stably expressed at an early fetal stage, suggesting that its expression from an early period is essential for the developing brain to function properly and that this protein has an important role, directly or indirectly, in creating neuronal networks in the developing brain, although the nature of their function at this developing stage of the human hippocampus remains unclear.

Acknowledgments

We would like to express our deep gratitude and appreciation to Prof Eguchi, Department of Pediatrics, Dokkyo University School of Medicine, Tochigi, Japan, and Drs Takashima and Itoh, National Institute of Neuroscience, Tokyo, Japan, for their valuable guidance and supervision. We are also grateful to Prof Montal, University of California at San Diego, for generously providing the complementary DNA of human *NMDAR1*.

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