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Expression of the gene coding for the NR1 subunit of the NMDA receptor during rat brain development

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Expression of the gene coding for the NR1 subunit of the *N*-methyl-D-aspartate (NMDA)-type of glutamate receptor was investigated in the developing rat brain. Peak NR1 gene expression in the whole brain occurred at approximately postnatal day (P) 10 with a second increase in the adult. To determine the ontogenic expression in the various brain regions, the expression of NR1 at P2, P10 and P60 was compared. The regional studies indicated increased expression at P60 in the cerebellum. In the midbrain and diencephalon, levels of expression at P10 and P60 were higher than at P2, while in the hippocampus, expression at P10 was significantly higher than at either P2 or P60. Expression in the other brain regions was constant over the period studied. These data indicate a region-specific expression of NR1 in the central nervous system during ontogeny.

The *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor is a ligand-gated ion channel which may play an important role in neuronal development, differentiation and plasticity in the central nervous system (CNS) [5, 6]. Activation of the NMDA receptor (NMDAR) has been implicated in synaptogenesis and in the formation of neuronal architecture, including the formation of optical dominance patterns in the visual cortex [28]. The NMDAR ion channel complex contains several pharmacologically distinct binding sites, including those for glutamate (or NMDA), glycine, polyamines, Mg²⁺, Zn²⁺ and open-channel blockers such as dizocilpine (MK801). All of these sites have been found to possess distinct ontogenic profiles which suggest that the regulation of NMDAR function may change during development. Recent studies have found NMDAR genes coding for four closely related subunits, NR2A–NR2D (or $\epsilon 1$ – $\epsilon 4$) and a single NR1 (or $\zeta 1$) subunit [14, 15]. The NR1 subunit isoform is the most abundant among seven distinct splice variants [24, 25]. The formation of a NMDAR with characteristic pharmacological properties requires a combination of NR1 and any one of the NR2 subunits [15, 17]. Furthermore, the pharmacological properties of the NMDAR such as affinity for agonists, antagonists and Mg²⁺ vary depending on the subunit composition [15, 17]. Expression of the NR1- and NR2-type subunits in the developing CNS has been partially

described using *in situ* and Northern hybridisation [29]. In the present work, we present quantitative measurements of regional changes in expression of the NR1 gene in discrete brain regions during ontogenesis.

Three litters of male Wistar rats were organised such that a representative of each litter could be sacrificed on postnatal day 0, 2, 5, 7, 10, 13, 17, 29, 39 and 66. To provide embryonic brain tissue 5 days before birth, three fetuses of embryonic day (E) 16 were pooled. Rats were sacrificed by decapitation and their brains immediately removed and frozen in liquid nitrogen. For regional studies, the hippocampus, cerebral cortex, cerebellum, diencephalon, midbrain, brainstem and corpus striatum (containing the caudate nucleus and putamen) were dissected from brains at P2 ($n = 4$), P10 ($n = 3$) and P60 ($n = 3$) as previously described [7].

Isolation of total brain RNA, electrophoresis and hybridisation were essentially as described elsewhere [13]. A 1-kb *EcoRI* fragment of an NR1 cDNA probe [17] kindly provided by Prof. Nakanishi (Kyoto University, School of Medicine, Kyoto, Japan) was labelled with [³²P]dCTP by random hexamer primers. To correct for interlane variability in the loading and transfer of RNA, blots were rehybridised with a ³²P-labelled oligonucleotide probe specific for 18S ribosomal RNA [11]. The levels of radioactivity from both hybridisations were determined directly from the blots using an AMBIS radioactivity counter (San Diego, CA).

Fig. 1 shows Northern blot analysis of NR1 subunit

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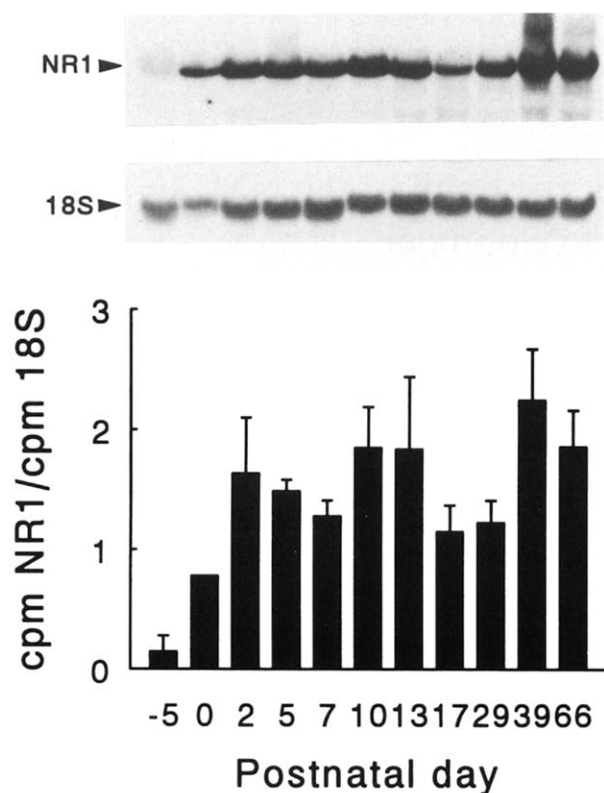


Fig. 1. The expression of the NR1 gene in rat whole brain between E16 and P66. An experiment consisted of Northern analysis of whole brain total RNA obtained from individual members of the same litter sacrificed at the times indicated. The experiment was repeated three times with animals from three separate litters. To account for differences between experiments resulting from minor variations in the specific radioactivity of the cDNA probe and washing of the filters in two experiments, counts from other time points were normalised by setting the counts at P0 to those obtained in the first experiment (actual values of cpm NR1/cpm 18S from the P0 time point of the three experiments were 0.78, 0.33 and 0.50). Data are presented as mean \pm S.D. of the normalised data from the three experiments. Differences were estimated by analysis of variance followed by Student–Newman–Keuls *t*-test.

mRNA in whole brain preparations. Expression was minimal in the foetus but increased rapidly after birth (P2 vs. E16 $P < 0.05$ analysis of variance followed by Student–Newman–Keuls *t*-test). A broad peak was present between P2 and P13. Expression decreased after P13 but increased again at P39 (P39 vs. P29 $P < 0.05$ analysis of variance followed by Student–Newman–Keuls *t*-test). Northern hybridisation of RNA from brain at P2, P10 and P60 (Fig. 2) revealed a constant level of mRNA in the cortex, brainstem and striatum. There was, however, a marked increase in expression in the cerebellum at P60 when compared to P2 and P10. In the hippocampus, expression of NR1 mRNA was maximal at P10. Similarly, expression in the midbrain and diencephalon was much higher at P10 than at P2, but in contrast to the hippocampus, NR1 mRNA levels remained relatively high in the adult.

The expression profile of NR1 mRNA correlates well, though not exactly, with the pharmacological analysis of NMDAR ontogeny. Binding of [3 H]MK801 to rat brain membranes is detectable as early as 3 days postnatal and is high in whole brain preparations between P14 and P28 [4, 16]. Further, L-[3 H]glutamate binding sites in the rat hippocampus are higher at P14 to P28 than in adults and similar transient increases in glutamate binding sites have been observed in the developing human hippocampus and kitten visual cortex [3, 8, 12, 21, 27]. Our data reveal sustained expression of NR1 in whole brain after a rapid increase between E16 and P7 (Fig. 1). Furthermore, expression of NR1 in the hippocampus was high at P10 (Fig. 2) in agreement with the reported peak of [3 H]glutamate binding [8, 27]. This suggests that the MK801 and glutamate binding sites may be partially regulated by the expression of the NR1 subunit in the neonatal brain. In contrast, the density of the [3 H]glycine sites associated with the receptor complex increases more slowly during development to reach a maximum in the adult [26]. This discrepancy between the temporal profiles of NR1 gene expression and the density of glycine binding sites could be explained by inclusion of NR2 subunits in the complex thus modifying the pharmacological properties of the receptor. In support of this concept, NMDAR complexes with combinations of NR1 and NR2C subunits, when expressed in *Xenopus* oocytes, exhibit reduced sensitivity to Mg^{2+} compared to combinations of NR1 and other NR2 subunits [15, 17]. Thus, hybrid receptor complexes may exhibit altered pharma-

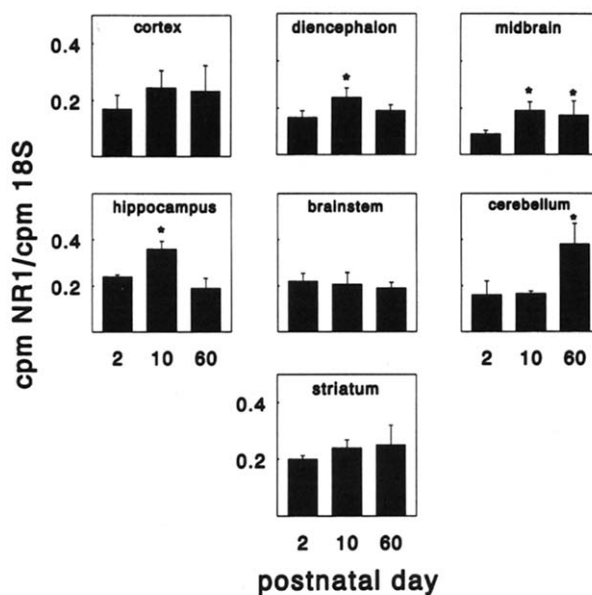


Fig. 2. Region-specific changes in expression of the NR1 gene from P2, P10 and P60. Data are expressed as mean \pm S.D. from individual animals. * $P < 0.05$. Differences were estimated by analysis of variance followed by Student–Newman–Keuls *t*-test.

ological properties during stages of development. Interestingly, the expression of the NR2C subunit, which is restricted to the cerebellum of the adult and the hippocampus at around P10 [15, 19], is coincident with high levels of NR1 mRNA (Fig. 2) and may indicate the requirement for a NR1–NR2C complex with particular pharmacological characteristics in the hippocampus of the neonate and the adult cerebellum.

The precise significance of the high NR1 expression in the hippocampus at P10 is unclear. A variety of evidence indicates that maturation of the rat hippocampus is completed at the end of the third week postnatal (reviewed in ref. 18). Furthermore, in the CA region of the rat hippocampus, the vast majority of excitatory synapses occurs on the dendrites of pyramidal cells which receive projections from granule cells of the dentate gyrus. Since the majority of the granule cells in the dentate gyrus is still undergoing neurogenesis after birth [1, 2, 22, 23], it is possible that the high NR1 expression in the hippocampus at P10 may underlie the mechanism of synapse formation between the granule cells of the dentate gyrus and the pyramidal cells of the CA region. The high levels of NR1 mRNA observed in the whole brain preparations between P10 and P14 (Fig. 1) may reflect the high level of expression in the hippocampus at this time. Similarly the high level of NR1 mRNA we detected in the adult cerebellum may contribute to the rise in expression found during maturation. The activity of the NMDAR in the developing cerebellum may be required for migration of granule cells into the internal granule cell layer from their site of neurogenesis in the external granule cell layer [10] and for synapse elimination between purkinje and climbing fibre cells [20]. The role of NMDAR in the adult cerebellum is unclear, however, it may be involved in long-term depression mediated by glutamate neurotransmission [9].

These data indicate a complex pattern of NR1 gene expression which may reflect changes in the ontogeny of binding sites. The different ontogenetic profiles of the binding sites raise the possibility that the NMDAR may undergo significant region-specific transcriptional or post-translational molecular organisation during development resulting in pharmacologically distinct receptors for NMDA.

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