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NMDA receptor 2C subunit is selectively decreased by MK-801 in the entorhinal cortex

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Abstract

Administration of the non-competitive NMDA receptor antagonist MK-801 (5-methyl-10,11-dihydro-5*H*-dibenzo[1,*d*]cyclohepten-5,10-imine) produces paradoxical neurotoxicity in limbic cortical regions which include the entorhinal cortex. The expression of NMDAR-2C but not -2A, -2B or -2D subunits was significantly decreased in rat entorhinal cortex layer III following MK-801 administration. These results suggest an important role for the NMDAR-2C subunit in the response to MK-801-induced neurotoxicity in brain regions highly vulnerable to injury.

Keywords: NMDA receptor; MK-801; Entorhinal cortex

Excess stimulation of NMDA/glutamate receptors plays a key role in pathophysiological processes such as neuronal damage in epilepsy, hypoxia-ischemia and neurodegenerative disorders. NMDA receptor antagonists have been developed which protect neurons from excitotoxic damage in the nervous system. In contrast to their protective effects on certain neurons, non-competitive NMDA receptor antagonists (e.g., 5-methyl-10,11-dihydro-5*H*-dibenzo[1,*d*]cyclohepten-5,10-imine (MK-801), phencyclidine, ketamine) have been found to produce paradoxical neurotoxic effects in some limbic brain structures, as evidenced by strong induction of mRNAs for c-*fos*, heat-shock protein 70, brain-derived neurotrophic factor (BDNF), and formation of intracellular vacuoles (Olney et al., 1989; Sharp et al., 1991; Castrén et al., 1993). These paradoxical neurotoxic effects have profoundly slowed the development of NMDA receptor antagonists as neuroprotective agents.

NMDA receptors are composed of homo- or heteromeric complexes of NMDAR-1 subunit and at least one of four NMDAR-2 subunits (NMDAR-2A, -2B, -2C, -2D) (Wafford et al., 1993). In the present study, the expression patterns of the four known NMDA receptor NMDAR2 subunits were investigated by in situ hybridization in a rat model of paradoxical neurotoxicity. The results obtained suggest a central role for alterations in NMDA subunit composition in response to neurotoxic injury.

Male Wistar rats were treated with saline or MK-801 (single dose i.p. injection, 5 mg/kg) and killed 4 h later. Oligonucleotides specific for NMDA receptor NMDAR-2 subunits were 36-mers constructed against cDNA sequences between TM1 and TM2:

NMDAR-2A, 5′-AGAAGGCCCGTGGGAGCTTTCCCTTTGGCTAAGTTT;

NMDAR-2B, 5′-GGGCCTCCTGGCTCTCTGCCATCGGCTAGGCACCTG;

NMDAR-2C, 5′-TGGTCCACCAGGTTTCTTGCCCTTGGTGAGGTTCTG;

NMDAR-2D, 5′-CGTGGCCAGGCTTCGGTTATAGCCCACAGGACTGAG.

Oligonucleotides were end-labelled with [^{35}S]dATP and in situ hybridization was performed on horizontal brain sections as previously described (Monyer et al., 1994). 14 μm thaw-mounted sections were fixed in 4% paraformaldehyde, hybridized in 50% formamide, 4 × standard saline citrate (SSC), 10% dextran sulfate and 10 mM dithiothreitol (~ 150 000 cpm/slide) at 42°C for 16 h, rinsed in 1 × SSC at 55°C for 30 min, dehydrated and exposed to film with ^{14}C standards for 28 days prior to data analysis.

Following MK-801 treatment the expression of the NMDAR-2C subunit, but not -2A, -2B, or -2D subunits, was significantly decreased (0.012 ± 0.001 vs. $0.004 \pm$

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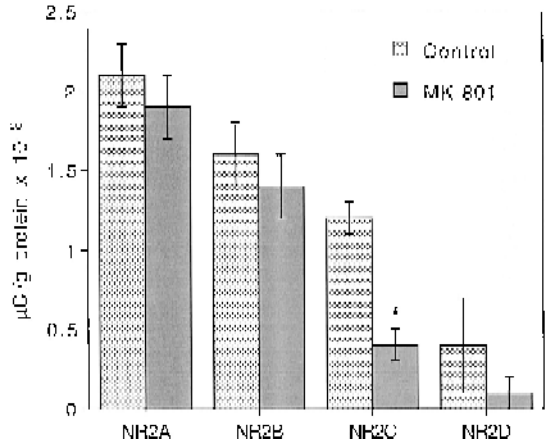

Fig. 1. In situ hybridization with specific oligoprobes NMDAR-2A (NR2A), NMDAR-2B (NR2B), NMDAR-2C (NR2C), NMDAR-2D (NR2D). Hybridized sections were apposed to β-Max Hyperfilm (Amersham, UK) for 28 days and values were obtained by image analysis (MCID M4, Imaging Research, Canada). Values are expressed in μCi/g protein. Data shown are means ± S.E.M. obtained from five (NR2A and NR2B) or three (NR2C and NR2D) brains performed in triplicate or duplicate. Bars representing control (stippled) or MK-801 treated (filled) are indicated. * $P < 0.05$.

0.001 μCi/g protein; $P < 0.05$, *t*-test) in the entorhinal cortex layer III (Fig. 1). Significant changes in NMDAR-2C subunit expression were not observed in the other entorhinal layers measured (layers II, V, VI) or in other brain regions (cingulate cortex, parietal cortex, temporal cortex, caudate putamen, thalamus, brain stem). Measurable amounts of NMDAR-2C labelling could not be observed in the hippocampus. As in the entorhinal cortex layer III, NMDAR-2C expression, but not -2A, -2B, or -2D expression, was significantly decreased in the internal granule layer (but not glomerular or external plexiform layers) of the olfactory bulb (0.037 ± 0.001 vs. 0.029 ± 0.002 μCi/g protein; $P < 0.05$, *t*-test). Significant differences in the expression of either NMDAR-2A or -2B subunits between control and MK-801-treated rats were not observed in any brain regions studied including the hippocampus (data not shown).

In the entorhinal cortex, MK-801 produces a strong increase in c-*fos* and BDNF mRNAs specifically in layer III neurons (Castrén et al., 1993). The entorhinal cortex layer III neurons also appear to be especially vulnerable in a rodent model of temporal lobe epilepsy or with excitotoxic chemical treatment (Du and Schwarcz, 1992; Du et al., 1995). Since the entorhinal cortex provides the major cortical input to the hippocampus, altering NMDA receptor subunit composition could influence learning, memory, and information processing functions (Jones, 1993).

Reduction of NMDAR-2C expression in response to MK-801 treatment is likely to change NMDA receptor composition towards receptors which contain NMDAR1 and NMDAR-2A and -2B subunits. This change will decrease the sensitivity of the NMDA receptors to glycine and glutamate 25- and 6-fold, respectively, since recombinant receptors composed of NMDAR1 and NMDAR-2C are more sensitive to these neurotransmitters (Wafford et al., 1993). If this is the case, regulation of NMDAR-2C subunits appears to be a response rather than a mechanism of selective vulnerability in layer III neurons. In this context, the possible impact of changes in subunit composition during neuropathological processes should be considered in the design of therapeutics aimed at specific NMDA receptor subtypes. Future studies should then be focused on the determination of mechanisms which render entorhinal cortex layer III more susceptible to neuropathological damage.

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