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MMP-2 and MMP-9 in Drug-Provoked Developmental Neuroapoptosis

V. Goranova[*], O. Uckermann[**], H. Luksch[**], J. Marzahn[**], Y. Hoehna[**], C. Ikonomidou[***]

[*]*Department of Anatomy, Histology and Embryology, Medical University, Varna, Bulgaria*
[**]*Department of Pediatric Neurology, University Children's Hospital, Dresden, Germany*
[***]*Department of Neurology, University of Wisconsin, Madison, USA*

Matrix metalloproteinases (MMPs) are zinc-dependent endoproteases with multiple roles in morphogenesis, cell death, and tissue regeneration. The aim of the present study was to investigate potential role of MMP-2/-9 in the pathogenesis of neuroapoptosis provoked by MK-801 or phenobarbital in the developing rodent brain. Seven-day-old rats or mice were drug-injected and pups were sacrificed at different survival times. Tissues from various brain regions were studied for expression of MMP-2/-9 by standard RT-PCR, western blotting, gelatin zymography and TUNEL immunohistochemistry. We found an increased number of TUNEL-positive cells 24 h after administration of MK-801 or phenobarbital. There was no significant increase in MMP-2/-9 mRNA expression, protein level or gelatinolytic activity observed in conjunction with drug-induced neuroapoptosis. The extent of neurodegeneration was not altered in MMP-9 TG rats and was increased in MMP-9 KO mice. Treatment with the broad metalloproteinase inhibitor GM6001 did not protect against drug-induced apoptosis. Our results suggest that activation of MMP-2/-9 does not contribute to pathogenesis of neuroapoptosis caused by NMDA antagonists or $GABA_A$ agonists in the developing rodent brain.

Key words: Matrix metalloproteinases, neuroapoptosis, MK-801, phenobarbital, GM6001.

Introduction

MMPs play an essential role in extracellular matrix remodeling and modulation of signaling pathways [4, 6]. They are known to be involved in the cleavage of cell surface receptors, release of apoptotic ligands, and chemokine-cytokine inactivation. MMPs are involved in various normal and pathological processes such as cell proliferation, migration, differentiation, angiogenesis, tissue repair, inflammation, tumor invasion, and apoptosis by degrading all kinds of extracellular matrix proteins and processing a number of bioactive molecules [10].

Our previous findings on the effects of N-methyl-D-aspartate (NMDA) antagonists, especially MK801 (dizocilpine), and $GABA_A$ agonist phenobarbital have shown that they cause a widespread neuroapoptosis in the infant mammalian brain [2, 3]. There exist data that MMP-9 control NMDA receptors [5].

The aim of the present study was to investigate potential involvement of selected MMPs in the pathogenesis of neuronal apoptosis induced by the NMDA antagonist MK-801 (dizocilpine) or the $GABA_A$ agonist phenobarbital in infant rats, transgenic (TG) rats overexpressing MMP-9 and MMP-9 knockout (KO) mice.

Materials and Methods

We used 7-day-old Wistar rats, MMP-9 TG rats and MMP-9 KO mice. Standard RT-PCR, western blotting, gelatin zymography and TUNEL immunohistochemistry were applied. The following drugs: 1 mg/kg MK-801 (dizocilpine), 50 mg/kg phenobarbital, 50 mg/kg GM6001 (ilomastat, a broad MMP inhibitor), dissolved in 0.1% DMSO 2 h later were i.p. injected. Fresh tissue frozen in liquid nitrogen from cortex, hypothalamus, and thalamus 1, 4, 12, 24, 48 or 72 h after injection of drugs were used for molecular/biochemical studies. For TUNEL peroxidase staining perfusion of brains with 4% paraformaldehyde in 0.1M PB under chloral hydrate anesthesia was performed 24 h after drug application followed by postfixation and embedding in paraffin. Paraffin sections were used for stereological quantification of neurodegeneration in different 13 brain regions evaluated in a blinded fashion.

Results

MK-801 and phenobarbital caused a widespread neuronal apoptosis throughout various regions of the immature rodent brain (**Fig. 1**). There is a more significant increase of cell death following treatment with MK-801 as compared to phenobarbital.

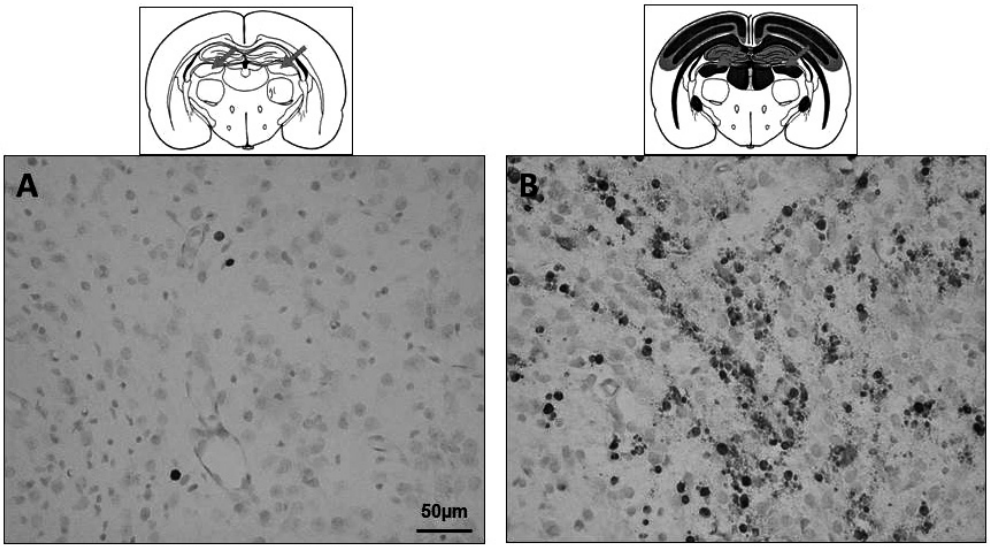

Fig. 1. Degenerated neurons detected by TUNEL staining in the laterodorsal thalamus (arrows) of control animals **(A)**, and 24 h after treatment with MK-801 **(B)**. DAB substrate, methyl green counterstaining

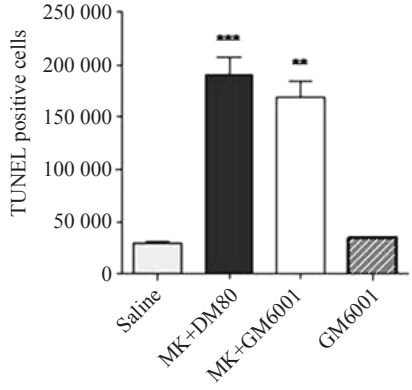

Fig. 2. Stereologic quantification of degenerating cells in the brain detected by TUNEL-staining 24 h after treatment with saline, MK-801 + 0.1% DMSO, MK-801 + GM6001 or GM6001. Values are means ± SEM. ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA and Tukey's post-hoc test, n = 6 per each group

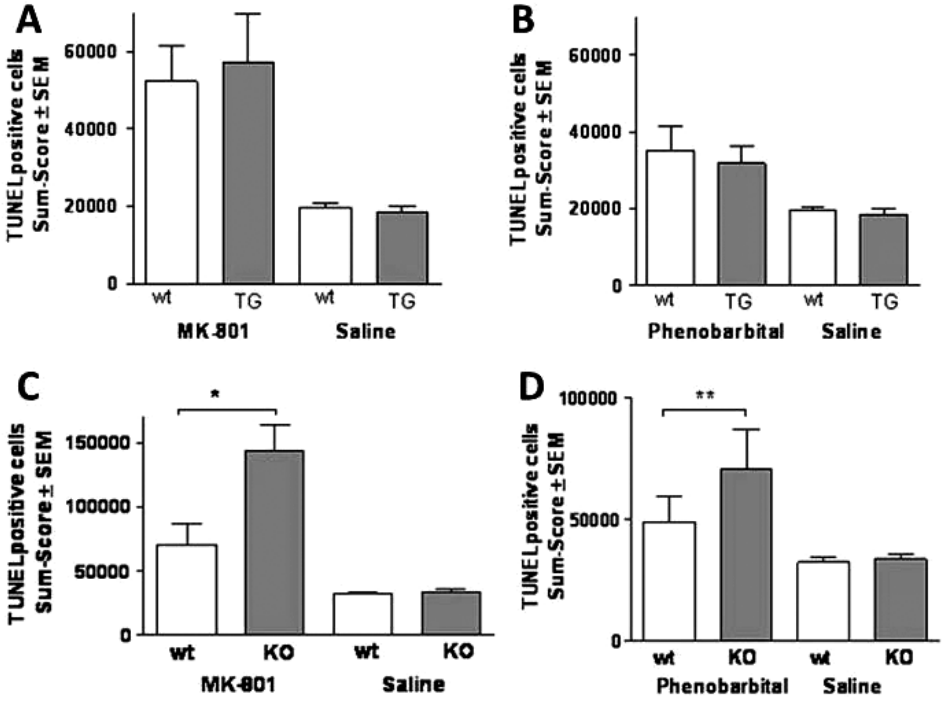

Fig. 3. Statistical analysis of the brain damage in TG rats and wild-type animals after treatment with MK-801 **(A)** or phenobarbital **(B)** and MMP-9 KO mice after treatment with MK-801 **(C)** or phenobarbital **(D)**. Values are means ± SEM. wt - wild type, TG - transgenic, KO - knockout, * $P < 0.05$, ** $P < 0.01$, unpaired t-test, n = 6 per each group

Stereologic quantification of degenerating cells in 13 brain regions detected by TUNEL-staining 24 h following treatment showed a significant increase of cell death. The application of GM6001 did not ameliorate the extent of brain damage induced by MK-801 (**Fig. 2**) or phenobarbital.

Drug-induced apoptosis was not altered in MMP-9 overexpressing rat brain. Statistical analysis revealed that the extent of brain damage indicated by number of TUNEL-positive cells was similar in transgenic rats and wild-type animals after treatment with MK-801 (**Fig. 3 A**) or phenobarbital (**Fig. 3 B**). We found a significantly enhanced drug-induced apoptosis in brains of MMP-9 KO mice for both drugs. The extent of neurodegeneration was statistically increased in MMP-9 KO mice than in brains of wild-type animals after treatment with MK-801 (**Fig. 3 C**) or phenobarbital (**Fig. 3 D**).

The expression of MMP-2 and MMP-9 were studied also at transcriptional, protein, cellular and functional level. No significant increase in MMP-2/-9 mRNA expression, protein level or gelatinolytic activity, observed in conjunction with drug-induced neuronal apoptosis, were found (data not shown).

Discussion

Our present findings confirm the effect of the NMDA antagonist MK-801 and $GABA_A$ agonist phenobarbital as potent neurotoxic agents that exert severe damage in the infant rodent brain. We did not found any morphological, molecular or biochemical data by the methods applied that MMP-2 and MMP-9 are pathogenetically involved in the developmental neuronal apoptosis induced by MK-801 or phenobarbital. The metalloproteinase inhibitor GM6001 does not prevent MK-801-induced neurodegeneration in the developing rat brain. Drug-induced brain apoptosis was not altered in MMP-9 TG rats but it was increased in MMP-9 KO mice compared to wild-type animals. This corresponded to previous data indicating a protective effect of MMP-9 after cerebral hypoxia-ischemia [9]. MMPs are important mediators in various neurodegenerative disorders [8] but they are known to contribute to neurogenesis and regeneration of the nervous system [1, 7, 11]. Additional experiments may reveal a potential beneficial or detrimental role of MMP-2/-9 or other members of the large family of MMPs in the immature brain.

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