NMDA receptors participate in control of manganese-enhanced MRI (MEMRI)

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INTRODUCTION

Mn^{2+} is known to behave like Ca^{2+} in many biological systems, and is also known to enter cells through calcium pathways such as voltage-gated calcium channels. Therefore, manganese-enhanced MRI (MEMRI) studies have been carried out extensively to visualize functional neural circuits and anatomy in the brain in vivo [1]. Despite the increase in interest in MEMRI, there have been few works addressing the involvement of voltage-dependent calcium channels in the rodent brain at the molecular level. There has been one report describing the expression of Fos protein, which is the marker protein after activation of voltage-dependent calcium channels [2], in relation to the contrast enhancement in MEMRI obtained for the same rats. In our preliminary experiment, we observed much lower contrast enhancement in MEMRI in rats anesthetized with ketamine, compared with other anesthetic drugs. Ketamine is known to be the noncompetitive antagonist for the NMDA receptor (NMDAR), which controls the influx of Ca^{2+} on binding to the excitatory neurotransmitter. Therefore, the contrast enhancement in MEMRI might be activity-dependently controlled by the NMDARs. The purpose of this study was to examine the effects of both NMDAR and AMPA receptor (AMPAR) antagonists on the contrast enhancement in MEMRI in rats administrated with Mn^{2+} ions.

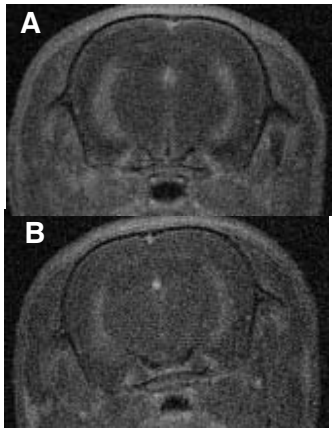
METHOD

***Paramagnetic Mn^{2+} Administration*:** Rats were intraperitoneally doped with paramagnetic Mn^{2+} ions, which were supplied from $MnCl_2$ (Sigma-Chemical Co. USA). Calcium-channel blockers, MK-801, NBQX, and CNQX (Terrios Pure Chemicals), were examined. MK-801 and NBQX were dissolved in aqueous PBS, and CNQX was dissolved in DMSO. NMDA (Wako Pure Chemicals) was prepared in saline and administrated intraperitoneally. Rats were anesthetized with ketamine (1-10 mg/kg), urethane (1.2 g/kg, Sigma) or nembutal (50 mg/kg, Dainippon Pharmaceutical). All drugs were administrated intraperitoneally at an injection volume of 1 ml/kg at least 20 min before anesthesia of examined animals. ***MRI measurements*:** MRI data were acquired using MRmini (MRTechnology, Tsukuba, Japan), consisting of a 0.5-Tesla permanent magnet made of Nd-Fe-B [3]. After appropriate positioning was confirmed on localizer images, axial, horizontal, and sagittal MR images were obtained using a T1-weighted 3D FLASH sequence. Typical imaging parameters were TR/TE/FA = 35/5.8/57º, matrix = 256 × 256 × 16, voxel size = 0.16 × 0.16 × 1.8 mm, NEX = 12.

RESULTS AND DISCUSSION

MR images taken after intraperitoneal $MnCl_2$ injection produced a clear signal enhancement in all examined brains of rats anesthetized with urethane or pentobarbital (Figure A). However, in rats anesthetized with ketamine, a remarkable inhibition of the signal enhancement in MEMRI was observed (Figure B). This inhibition was dependent on the amount of ketamine used for rats as an anesthesia. Since ketamine is known as a noncompetitive NMDAR antagonist, these results might suggest that ketamine blocked Mn^{2+} influx through NMDAR. Thus, the effects of both NMDAR and AMPAR antagonists, MK-801, CNQX, and NBQX, on MEMRI were examined. When rats were treated with the most potent of these antagonists, MK-801, the signal enhancement by Mn^{2+} was remarkably inhibited. Treatment of rats with NBQX failed to inhibit the enhancement of the signal intensity in MEMRI. In contrast, the less selective AMPAR antagonist, CNQX, prevented the enhancement of the signal intensity in MEMRI, indicating that the calcium channel in AMPAR does not contribute to the influx of Mn^{2+} observed in MEMRI studies. The MEMRI results obtained with both NMDAR and AMPAR antagonists suggest that the signal enhancement in MEMRI in rat brains is affected by NMDAR, but not AMPAR. In conclusion, this is the first report to demonstrate the effects of calcium-channel blockers on MEMRI in rat brains, although it has been shown at cellular levels.

T1-weighted MR Images of the rat head after 2 h $MnCl_2$ administration.



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