Intratympanic manganese administration revealed sound intensity and frequency dependent functional activity in rat auditory pathway

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Introduction

While it is well known that the middle ear is unique in its ability to transmit sounds to the cochlea, the biomechanical basis for sound transmission at various frequencies is poorly understood, which has clinically important consequences. More specifically, while it is believed that the middle ear plays a key role in sound transmission, it is largely unknown that how the sound at different frequencies propagates from middle ear to central auditory system. The imaging techniques such as MRI and CT are helpful in providing an adequate mophologic pictures of middle ear but neural activity related with sound propagation cannot be evaluated by these conventional imaging techniques. Manganese-enhanced MRI (MEMRI), which makes use of the fact that paramagnetic manganese ions (Mn^{2+}) enter synaptically activated neurons through voltage-gated calcium channels and results in signal enhancement on T1-weighted MRI images, is increasingly used for the functional characterization of the auditory system. In this study, using MEMRI with intratympanic administration, we investigated how the sound at different frequencies propagates from middle ear to central auditory system. Furthermore, we also investigated the possible relationship between sound intensity and auditory neural activity.

Material and Methods

Rats were anesthetized using 5% isoflurane (Choongwae Pharma. Corp., Seoul, Korea) and were injected with manganese chloride (0.25 mmol/kg body weight) dissolved in distilled water via intratympanic injection sites. For the intratympanic administration, the injections were performed under inhalation anesthesia by inserting a 30-gauge needle into the tympanic membrane. To be consistent across animals, only the bevel of needle (about 1.5 mm) was inserted into the tympanic membrane with the aid of a microscope in all animals. The animals were divided according to frequency stimulation. first is none stimulate, second is 10 kHz stimulate and last is 40 kHz stimulate. All sound is about 90 decibel. Imaging was performed using a 9.4T VNMRS horizontal bore scanner (Varian Inc., Palo Alto, CA, USA). A 72-mm inner diameter volume coil (Rapid Biomedical GmbH, Rimpar, Germany) was used for RF transmission and the signal was received using a 4-channel array head coil (Rapid Biomedical). MR imaging was acquired 2D spin-echo T1-weighted image and sequence were as follows: field of view, 36×27 mm matrix size, 256×256 axial slices, 0.5 mm slice thickness, no gap, repetition time (TR) = 600 ms, echo time (TE) =13.82 ms, number of acquisition (NEX)=6, scan time = 11m 34s. The animals were placed in the magnet in a prone position with the head first. During MRI measurements, Each animal was anesthetized by isoflurane (5% induction, 1.5% maintenance) with the mixed gas of N_2O (70%) and O_2 (30%). Respiration and body temperature were monitored and maintained using circulating warm air and MR compatible small animal monitoring and gating system (SA instruments, Stony Brook, NY, USA).

Results

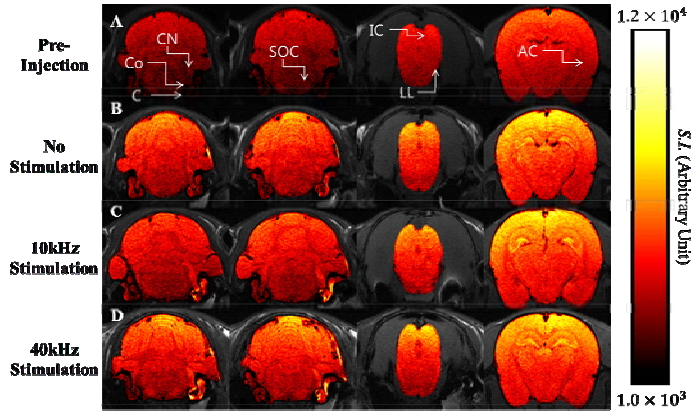

Figure 1. T1-weighted MR images of auditory pathway of rat before (A) and 24 hours after intratympanic Mn^{2+} administration (B, C, D). Abbreviations: cochlea (C), cochlear nerve (Co), cochlear nucleus (CN), superior olivary complex (SOC), lateral leminiscus (LL), inferior colliculus (IC) and auditory cortex (AC).

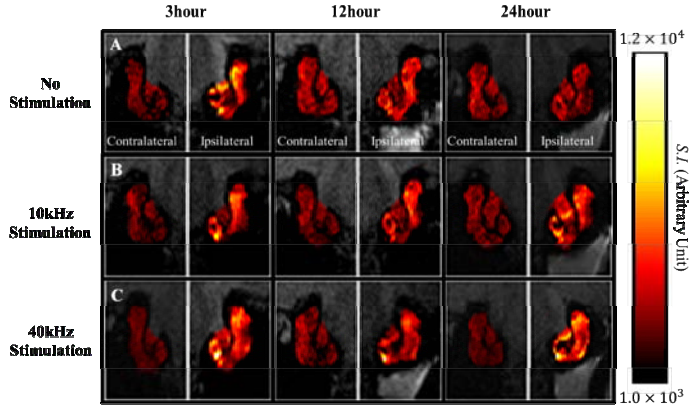

Figure 2. Cochlear enhancement at 3, 12 and 24 hours after Mn^{2+} administration in case of (A) without sound stimulation, (B) 10 kHz stimulation and (C) 40 kHz stimulation. Compared with sound stimulation, the signal enhancement of the cochlea without stimulation showed a much lower and more sparse signal enhancement at 24 hours after Mn^{2+} administration.

Figure 1 shows a clear delineation of the structures in the primary auditory pathway and frequency-dependent differences in signal enhancement 24 hours after intratympanic Mn^{2+} administration by injection into the middle ear cavity of a normal rat. The enhanced auditory structures were the cochlea (c), cochlear nerve (Co), cochlear nucleus (CN), superior olivary complex (SOC), lateral lemniscus (LL), inferior colliculus (IC) and auditory cortex (AC). The caudal surface of the inferior colliculus was chosen as a reference plane following the histological convention for the rat auditory system. The cochlea showed strong enhancement at an earlier time (three hours after intratympanic administration of Mn^{2+}), however, a continuous decrease in enhancement was observed after six hours (Figure 2). That is, inside the cochlea, Mn^{2+} passed through the round window membrane and was distributed from the perilymphatic space to the entire cochlea a short time after intratympanic administration, but Mn^{2+} had been removed from the cochlea within six hours after intratympanic administration. However, in rats who received sound stimulation (10 kHz and 40 kHz), auditory structures showed gradual signal enhancement after intratympanic administration of Mn^{2+}. Compared to 10 kHz, sound stimulation of 40 kHz showed stronger enhancement at all time points.

Conclusion

We demonstrated differential manganese signal enhancements according to sound intensity and frequencies in the ascending auditory pathway of the rat after administration ofintratympanic$MnCl_2$. Compared to signal enhancement in rats who did not receive explicit sound stimuli, auditory structures in the ascending auditory pathway showed stronger signal enhancement in rats who received 10 and 40 kHz sound stimuli. Furthermore, the signal enhancement with a stimulation frequency of 40 kHz was stronger than that with 10 kHz. These results seem to suggest that pooled neural activity from many auditory neurons or higher firing rate from auditory neuron is necessary for achievement of a response to high sound intensity and/or frequency. Finally, results of the current study suggested that MEMRI by intratympanic administration may provide a new and convenient in vivo imaging tool for attainment of a more complete understanding of important but poorly understood biomechanical mechanisms for the way in which sound at different frequencies and intensities is propagated from the cochlear to central auditory centers.