Introduction
It is known that global ischemia causes neuronal death in the selectively vulnerable CA1 region of the hippocampus. This histopathologic neural damage is explained by the combined effects of glutamate over-excitation and free radical toxicity (1). Functional and morphological in-vivo evaluation of hippocampus is important to estimate memory disturbance after ischemic disease such as stroke or myocardial infarction. However, hippocampal function has been difficult to visualize in-vivo because the structure is complicated, and there are no specific contrast enhancements on MRI, CT, and PET. Recently, it has been reported that manganese-enhanced MRI (MEMRI) can enhance the hippocampal CA1-3 formation and dentate gyrus (DG) selectively at least one day after systemic intravenous MnCl$_2$ administration (2, 3). Manganese is known that it can shorten T$_1$ relaxation time and enhance T$_1$-weighted MRI. Blood-CSF barrier can uptake manganese via choroid plexus and transport it to the DG and hippocampus directly. Therefore, MEMRI can distinguish the hippocampal formation in the intact rat brain. However, there is as yet no report demonstrating whether MEMRI can observe the neuronal damage in the hippocampus. Accordingly, we investigated the hypothesis that transient cardiac arrest using a short-acting β-blocker in the rat would alter MEMRI enhancement due to neuronal damage in the hippocampus. Furthermore, we improved the cardiac arrest method of Liachenko et al (4) by using only autologous blood without the need for donor’s blood.

Materials and Methods
12 male Wistar rats (300-350 g) were used. Two groups of rats were used including sham control (n = 4) and rats 4 weeks after cardiac arrest (n = 4). The cardiac arrest model was prepared using a modified technique of the model described by Liachenko et al. (4). Rats were initially anesthetized with 4.0 % isoflurane, and then anesthesia was maintained with 1.5-2.0 % isoflurane mixed with a 1:2 O$_2$ / room air gas mixture using a rodent ventilator. Rectal temperature was maintained at 37.5 ± 0.2 ºC using an auto-controlled heart pad. Polyethylene catheters (PE-50, Becton Dickinson) were placed in both the femoral artery and left femoral vein for drug administration, blood pressure monitoring and blood gas measurements. After collection of arterial blood (5 mM/kg), the muscle relaxant vecuronium (1 mg/kg, Sankyo) was administrated to suppress any spontaneous respiration. Then, 25 mg/kg esmolol (short-acting β-blocker, Baxter) was administrated IV to induce cardiac arrest. After confirmation that the blood pressure decreased and the heart beat stopped, time keeping was started. Artificial ventilation and heat pad were shut off during cardiac arrest. Arterial blood withdrawn from the animal was re-administered with added epinephrine (0.024 mg/kg), sodium bicarbonate (17.64 mg/kg), and heparine (5 U/ml) 3 minutes after starting the timekeeping. Typically, blood pressure and heart beat were recovered 5 minutes after the esmolol induced cardiac arrest. For all groups, 252.7 µmol/kg MnCl$_2$ (Sigma) was given by infusing 75 ml/kg of a 50 mM MnCl$_2$ at a rate of 2.5 ml/hour through the tail vein (2). During MnCl$_2$ infusion, the anesthesia was kept light between 0.8-1.5 %. After the MnCl$_2$ administration, animals were kept in an incubator (30-32 ºC) for 24 hours to maintain body temperature. MnCl$_2$ was administered four weeks after cardiac arrest was performed. The MRI acquisitions were performed 3 days after the manganese administration on a 4.7-T horizontal MRI (CSI-II-Omega, Bruker). A 30 mm Litz coil (Doty Scientific) was used. The measurements were performed in the following order: T$_1$-weighted (T1W) MRI, DWI (b = 1000), and T$_2$-weighted imaging. Multi-slice T1W coronal and horizontal images were obtained using a spin-echo sequence with the following parameters: pulse repetition time (TR) = 250 ms, echo time (TE) = 10 ms, matrix size = 256 × 256, field of view (FOV) = 32 × 256, field of view (FOV) = 32 mm, slice thickness (ST) = 1.2 mm, number of acquisitions (NA) = 18, number of slices = 8, and acquisition time for one set = 19.2 minutes. One complete set of T1W measurements consisted of two T1W scans with the offsets of 0mm and 1mm to maintain continuity of slices and to cover entire volume. T2W and DW2 were obtained using a spin-echo sequence with the same slice orientation as the MEMRI with the following parameters: TR = 2500 ms, TE = 80 ms, matrix size = 256 × 256, FOV = 32 mm, ST = 1.2 mm, NA = 2, number of slices = 8, and acquisition time for one set = 46 minutes.

Results and Discussion
There were two major findings in this study: 1) Signal was not enhanced significantly in the hippocampal CA1 region in the cardiac arrest group on the T1W MRI (Fig. A). Signal intensity in CA1 was decreased in the cardiac arrest model in comparison with CA3 (Fig. B). In contrast, significant signal enhancement was observed in the CA1 region in the sham control group and CA3 region in both the sham and cardiac arrest group (P < 0.05, Bonferroni/Dunn test, Fig. C). 2) Histopathologic neural damage was observed at CA1 region in the cardiac arrest group (Fig. D). An improved cardiac arrest model using only autologous blood provided transient global ischemia and selective neuronal damage in the hippocampal CA1 region. These results confirm that MEMRI can provide visualized detection of the hippocampus and furthermore MEMRI is sensitive to hippocampal neuronal damage even four weeks after a short (5 min) ischemic event. We speculate that the changes in hippocampal enhancement measurement with MEMRI reflects a functional deficit. In addition, we have improved the cardiac arrest model using only autologous blood enabling investigation of the relationship between ischemic disease and blood proteins such as neuro-protective protein, tolerance, and apoptosis.

References