Magnetic Resonance Imaging-Microdialysis Simultaneous Measuring System* (Neurotransmitter Levels in a Rat Brain during Magnetic Resonance Imaging)

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A new simultaneous measuring system using Magnetic Resonance Imaging (MRI) and microdialysis was developed. Because MRI and microdialysis have complementary measuring advantages, the combination of the two methods can provide less invasiveness, high detection sensitivity and multimodality for in vivo biomedical measurement. Using the MRI-Microdialysis simultaneous measuring system, preliminary experiments were performed to examine the rapid effect which MRI has on rat brain metabolism and to analyze the acute physiological response of the brain to oxidative stress. The changes in 5-hydroxyindoleacetic acid (5HIAA), noradrenaline levels in the striatum and 5HIAA, L-aspartic acid and L-asparagine levels in the hypothalamus area were not significant when compared to normal changes in the absence of the MRI sequence. There were no significant changes in 5-HIAA and glutamine levels during the oxidative stress; however, the intensity of the MR image was increased. The increase suggested that a decrease in paramagnetic molecules occurred during the oxidative stress period in the brain.

Key Words: Biological Engineering, Medical Engineering, Electromagnetic Measurement, Magnetic Resonance Imaging, Microdialysis, Rat Brain, Neurotransmitters, Oxidative Stress

1. Introduction

Magnetic Resonance Imaging (MRI) is a useful noninvasive measuring technique for interdisciplinary studies such as neurological biomechanics. Functional MRI (blood oxygenation level dependent functional MRI, BOLD-fMRI) has made it possible to determine the activation of neurons during a specific biomechanical task such as hand motion control in human beings⁽¹⁾. Although MRI offers many advantages, there is a limitation in regard to what substances can be measured. For instance, it is impossible to detect n~pM ranges of neurotransmitters in the brain of living animals⁽²⁾. Although MRI is a noninvasive technique, it requires some special physical conditions for carrying out the measurements. These are a

strong magnetic field (0.5~2.0 T, 1 T=10 000 G), a dynamic magnetic field and radio waves. Little knowledge is available on the influence of the measurement environment of MRI on brain metabolism.

Microdialysis is a technique to monitor the chemistry of the extracellular space in living tissues, such as the brain⁽³⁾. The microdialysis probe is designed to mimic a blood capillary. When artificial cerebrospinal fluid (CSF) is slowly pumped by a microinjection pump through the microdialysis probe, the solution becomes equilibrated with the surrounding extracellular tissue fluid. It then contains a representative proportion of the tissue fluid's molecules⁽⁴⁾. Although microdialysis is an invasive measuring technique, it has a very high detection sensitivity for neurotransmitters such as monoamines (nM to pM) because it uses an electrochemical detector.

Because MRI and microdialysis have the complementary measuring advantages described above, a simultaneous measuring system using MRI and microdialysis was developed. It can provide less invasiveness, high detection sensitivity and

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multimodality for in vivo biomedical measurement. We present 1) the behavior of neurotransmitters in a rat brain during MRI to consider the influence of MRI on brain metabolism, and 2) the acute physiological response of the brain to oxidative stress (100% oxygen exposure, hyperoxia), using the MRI-Microdialysis simultaneous measuring system.

2. Materials and Methods

2.1 MRI-Microdialysis simultaneous measuring system

The MRI-Microdialysis simultaneous measuring system consists of three units; the first is an MRI apparatus (2.0 T GE Omega CSI imager), the second is a microdialysis unit (CMA100, 140, CMA) with high performance liquid chromatography with an electrochemical detector (LC4C, BAS) and a fluorescence detector (CMA280, CMA). The third unit consists of animal care apparatuses, such as a body temperature controller (FC301, IUCHI), a small animal anesthesia apparatus (SN485, SHINANO) and a portable clinical analyzer (i-Stat, i-Stat Co.). Figure 1 indicates the schematic layout of this system.

MRI is based on nuclear magnetic resonance (NMR). By applying a magnetic field and radio frequency waves, NMR detects the motion of the

magnetic moment in the individual nucleus. MRI can image the spatial distribution of nuclei, relaxation times and other observable values noninvasively⁽²⁾. New methods such as 3D diffusion imaging sequences and ultrafast 3D imaging sequences have been developed^{(5),(6)}. In the present experiment, the widely used ¹H Spin Echo (SE) imaging method and ¹H Gradient Echo imaging method were used as standard techniques⁽⁷⁾.

Microdialysis is an invasive measuring technique, but it has a very high detection sensitivity for neurotransmitters such as monoamines (nM to pM) as described before. MRI and microdialysis have complementary measuring advantages.

2.2 Experimental procedure

2.2.1 Animal and experimental preparation

6-42 week old male Wistar rats ($n=6$) were used for the experiment. The rats were anesthetized with pentobarbital sodium (50 mg/kg), and their body temperature during surgery was maintained by a heating blanket (CMA150). Their skulls were exposed and a 1 mm hole was drilled through the bone above the area to be perfused. An intracerebral guide cannula (BAS) was implanted into the striatum (-0.8 mm anterior to the bregma, 3.9 mm lateral to the middle suture and 3.7 mm ventral to the dura, Fig. 2)⁽⁸⁾ or into the hypothalamus (-0.4 mm anterior to the bregma, 1.6 mm lateral to the middle suture and 7.6 mm ventral to the dura) according to the stereotaxic coordinate⁽⁹⁾. The guide cannulas were fixed permanently with acrylic resin. The surgery for the intracerebral guide cannula setup was performed at least three days before the experiment. During the magnetic resonance and microdialysis experiment, the rats were anesthetized with isofluorene/N₂O/O₂ gas (with urethane 0.75 g/kg for oxidative stress analysis) and their body temperature was controlled using a circulating water blanket maintained at 37°C. A nonmetal microdialysis probe (0.5 mm in diameter with a 2 mm membrane length for the striatum, 20 000 daltons cut off, BR2, BAS) was implanted into the guide cannula at least 3 hr before the experiment. The microdialysis probe was connected to a microinfusion pump via a 2.5 m microtube. The region was continuously perfused at a constant rate of 2.0 μ l/min with synthesized CSF (NaCl 145 mM, KCl 3 mM, CaCl₂ 1.2 mM, MgCl₂ 1.2 mM, NaH₂PO₄ 0.67 mM and Na₂PO₄ 2.33 mM). Samples were collected every 5~10 min with a microinfusion pump and a fraction collector. Neurotransmitters were determined with high performance liquid chromatography (HPLC) using an electrochemical detector (LC4C, BAS) and a fluorescence detector (CMA 280, CMA).

Fig. 1 Scheme of designed MRI-Microdialysis simultaneous measuring system

2.2.2 Neurotransmitter levels in a rat brain subject to MRI

To consider the influence of MRI on brain metabolism, sample collection using microdialysis from the rat brain was performed outside the MR magnet for about 45 min, after which the rat was moved into the MR magnet, and MRI and microdialysate collection were performed continuously for about 60 min. Neurotransmitter monitoring was continued for another 60 min after the MR Imaging outside the magnet. MRI was performed on a 2.0 T GE Omega CSI imager. The animals were imaged using a 150 mm diameter ^1H birdcage-type imaging coil. Spin-echo images were acquired with a repetition time of 1 000 msec, an echo time of 25 msec, and a field of view with a 64 mm $\times$ 64 mm and 256 $\times$ 256 matrix size. The slice thickness was 2 mm.

2.2.3 Acute physiological response of the brain to oxidative stress

To consider the acute physiological response of the brain to oxidative stress, rats were exposed 100% oxygen for about 30 min during MRI. Sample collection from the rat striatum using microdialysis was performed before, during and after the oxidative stress. The animals were also MR imaged using a 90 mm diameter ^1H birdcage-type imaging coil. T_2 -weighted gradient echo images were acquired with a repetition time of 500 msec, an echo

time of 30 msec, and a field of view with a 50 mm $\times$ 50 mm and 128 $\times$ 128 matrix size. The slice thickness was 2 mm.

3. Results

3.1 Neurotransmitter levels in a rat brain subject to MRI

Neurotransmitters in a rat brain during MRI were measured using a MRI-Microdialysis simultaneous physiological measuring technique to consider the influence of MRI on brain metabolism. Several changes were detected in the neurotransmitters by this technique. Figure 3 indicates the typical relative changes in 5-HIAA (5-hydroxyindole acetic acid) and NA (noradrenaline) in the rat striatum during 45 min outside the MR magnet, 60 min of MR imaging, and another 60 min outside the magnet. About 2.7 hr of data are shown. The relative values of 5-HIAA and NA changed continuously. The change was within the range of +15 $\sim$ -10% for 5-HIAA and 0 $\sim$ -30% for

Fig. 2 Magnetic resonance image of a rat and location of microdialysis probe in the rat striatum (axial image)

Fig. 3 Relative change of 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) release in the rat striatum during magnetic resonance imaging

Fig. 4 Relative change of 5-hydroxyindoleacetic acid (5-HIAA) release in the rat hypothalamus area during magnetic resonance imaging

Fig. 5 Relative change of amino acid release in the rat hypothalamus area during magnetic resonance imaging (ASP: L-aspartic acid, ASN: L-asparagine)

NA. Figure 4 shows the change in 5HIAA release in the rat hypothalamus area. About 2.5 hr of data are shown. The levels decreased continuously at the rate of 0.16% / minute on average. Figure 5 indicates the change in L-aspartic acid (ASP) and L-asparagine (ASN) levels in the rat hypothalamus area. During the experiments, the monoamine and amino acid levels in the rat brain changed continuously. The changes were not significant when compared to normal changes in the absence of the MRI sequences for the present results.

3.2 Acute physiological response of the brain to oxidative stress

Neurotransmitter analysis and T_2 -weighted gradient echo image collection were performed simultaneously using a MRI-Microdialysis simultaneous measuring system to consider the acute physiological response of the rat brain to oxidative stress. Figure 6 shows the relative change in 5-HIAA, glutamine (GLN) in the rat striatum and gradient echo images. 5-HIAA and GLN levels changed continuously, but there was no strong correlation between the changes and the oxygen exposure. Figure 6 shows the MR images collected before (A), during (B) and after (C) the oxidative stress. Signal intensity in the MR image in the oxygen exposure period increased as shown in Fig. 6 (ΔBA , ΔCB).

4. Discussion

4.1 Evaluation of the measuring technique

Many monoamines exist in the striatum or hypothalamus area. Dihydroxyphenyl acetic acid (DOPAC), homovalinic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) can easily be detected by microdialysis and electrochemical detectors. To avoid the influence of the magnetic field, the micro-

Fig. 6 Relative change of 5-hydroxyindoleacetic acid (5-HIAA) and glutamine (GLN) release in the rat striatum during oxidative stress (100% O_2 exposure), and change of T_2 weighted MR image of the rat brain (axial image, A: before, B: during, C: after oxidative stress)

injection pump and the fraction collector were located 2-3 meters away from the MR magnet. It took about 12-15 min to collect the dialysate from the brain (2 μ l/min). It was necessary to use acetic acid as an antioxidizing agent. Temperature control of the dialysate at 4°C is also helpful for the antioxidization of the dialysate. On the other hand, because amino acids such as L-aspartic acid (ASP), L-asparagine (ASN) and glutamine (GLN) are more stable than monoamines, they were easily detected by microdialysis and by using a fluorescence detection.

4.2 Neurotransmitter levels in a rat brain subject to MRI

Neurotransmitter levels were analyzed using the microdialysis method during MRI in two areas of the rat brain. One was the striatum, and the other was the hypothalamus. The striatum plays a principal role in motion control. It is one of the important research

areas for the interdisciplinary study of neurological biomechanics for the future. On the other hand, the hypothalamus plays an important role as the main center of the autonomic nervous system. The hypothalamus area may also have sensitivity to the electromagnetic field. If there is any influence of the MR imaging environment, such as a strong magnetic field of 2 T (20 000 gauss), a dynamic magnetic field and radio frequency waves, some changes may be indicated in the area. 5HIAA is a metabolite of serotonin (5HT, hydroxytryptamine). 5HT decreases activity, and enhances inactivity. It is one indicator of neural activity. Noradrenaline (NA) is biosynthesized from tyrosine. NA increases in the rat brain under stress conditions. It is one indicator of stress level in the rat.

In the preliminary experiments, the change of 5HIAA and NA levels in the caudate putamen and 5HIAA, ASN and ASP levels in the hypothalamus were not significant compared to the normal changes in the absence of the MRI sequence. It has previously been reported that a magnetic field (50 mT, 60 Hz) did not affect the serotonin release under restraint stress in the mouse brain, though it appeared to suppress the serotonin turnover rate because of the accumulation of serotonin⁽⁹⁾. However, experimental conditions such as the magnetic field for the mouse and for the rats used in this study were different. This MRI sequence and experimental procedure may not have had a significant direct influence upon the monoamine and amino acid levels in the rat brain⁽¹⁰⁾. If MRI has any influence on the brain metabolism, not only on monoamine and amino acid levels in the rat brain but also on any other metabolism, it may appear after a long time or under high RF power MRI or due to some other monitoring methods. For further understanding, consideration of the measuring substances and statistical analysis are necessary.

4.3 Acute physiological response of the brain to oxidative stress

Preliminary experiments were performed to consider the acute physiological response of the brain to oxidative stress (normobaric 30 min 100% oxygen exposure, hyperoxia). Neurotransmitter analysis and T₂ weighted gradient echo image collection were performed simultaneously using an MRI-Microdialysis simultaneous measuring system. It has previously been reported that, after hyperbaric O₂ exposure in excess of 6.0 atmospheres absolute in 20 min, the cerebral pools of glutamate, aspartate, and γ -aminobutyric acid (GABA) decreased significantly while the glutamine content increased relative to the control⁽¹¹⁾. In our study, 5-HIAA and GLN levels changed continuously and there was no strong correlation between the changes and the oxygen exposure.

Normobaric oxygen exposure (100%, 30 min) caused no significant change in neurotransmitter levels in a rat brain subject to oxidative stress, though longer oxygen exposure (48 hrs) under normobaric condition caused changes in synaptic function in rats⁽¹¹⁾. However, biochemical responses in the rat brain were not significant, the oxygen stress increased the signal intensity in the MR images as shown in Fig. 6 (Δ BA, Δ CB). The changes in the vascular content of paramagnetic molecules such as deoxyhemoglobin changed the intensity of the MR images. Because an increase in deoxyhemoglobin which was elevated in anoxia produced decreased signal, Fig. 6 (Δ BA, Δ CB) suggested that a decrease in paramagnetic molecules occurred in the oxidative stress period in this experiment.

4.4 Prospects of the MRI-Microdialysis simultaneous measuring system

Microdialysis is useful for analyzing not only monoamines and amino acids but also other metabolites such as glucose and lactates by optimum detection techniques. It is also possible to introduce an exogenous compound into the brain during MRI. Thus, microdialysis is a complementary measuring technique for noninvasive MRI. A simultaneous measuring system including MRI and microdialysis will open up a new era of biomedical research in the future, due to the following:

In MRI, the water content and the water relaxation times T₁ and T₂ are used to generate tissue contrast. In addition to anatomical imaging, functional imaging has been developed in which dynamic properties such as water diffusion, brain blood flow, blood volume and oxygenation have been used for image contrast. The metabolic background of the magnetic resonance image signals can be analyzed using the MRI-Microdialysis simultaneous measuring system.

Magnetic resonance spectroscopy (MRS) or magnetic resonance spectroscopic imaging (MRSI) is a very powerful technique for studying brain chemistry in vivo. Proton (¹H), phosphate (³¹P) or carbon (¹³C) MRS or MRSI offer valuable biochemical information. Using ¹H MRS, N-acetylaspartate, choline-containing compounds, total creatine compounds, glutamine, glutamate, L-lactate, γ -aminobutyric acid (GABA) and D-glucose can be detected⁽¹³⁾. ³¹P MR spectroscopy contains information about the energy state of tissues, phosphocreatine and inorganic phosphorus. Although it is a powerful method, MRS is inherently limited by its low sensitivity for in vivo studies. Metabolite levels in the mM range can be imaged at less than 1 ml in vivo⁽²⁾. Microdialysis has the advantage of high detection sensitivity. It can

detect a nM to pM order of metabolites. It is $10^3 \sim 10^6$ times more sensitive than MRS. The metabolic background of MR spectroscopic signals can be analyzed using this simultaneous measuring system. This will contribute not only to in vivo biochemical research but also to the development and evaluation of new MR techniques for spectroscopy (MRS) or spectroscopic imaging (MRSI).

5. Conclusions

A simultaneous measuring system with magnetic resonance imaging (MRI) and microdialysis was designed and studied. We presented the behavior of neurotransmitters in a rat brain during MRI to consider the influence of MRI on brain metabolism. The changes in 5HIAA and NA levels in the striatum and 5HIAA, ASP and ASN levels in the hypothalamus area were not significant when compared to the normal changes in the absence of MR sequences in preliminary experiments.

Neurotransmitter analysis and T_2 -weighted gradient echo image collection were performed simultaneously using the MRI-Microdialysis simultaneous measuring system to consider the acute physiological response of the rat brain to oxidative stress. There were no significant changes in 5-HIAA and glutamine levels subject to the oxidative stress; however, the intensity of the MR image was increased during the oxidative stress period. The intensity change suggested that a decrease in paramagnetic molecules was occurred during the oxidative stress period in this preliminary experiment.

Magnetic resonance imaging/spectroscopy (MRI/S) and microdialysis have complementary measuring advantages. The combination of the two methods can provide less invasiveness, high detection sensitivity and multimodality for in vivo biomedical measurement. The MRI-Microdialysis simultaneous measuring system will contribute greatly to biological and medical engineering such as developing and evaluation of new MRI/S techniques, and to the understanding of in vivo biomechanisms.

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