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CEREBRAL OXYGEN UTILIZATION ANALYZED BY THE USE OF OXYGEN-17 AND ITS NUCLEAR MAGNETIC RESONANCE

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SUMMARY: In order to assess the usefulness of oxygen-17, a stable isotope of oxygen, oxygen-17 was administered to rats for studying cerebral oxygen utilization, and the produced metabolic water was detected by ^{17}O-NMR spectroscopy in vitro and an ^{1}H-NMR imaging system in vivo. In the vitro study, the increment in signal amplitude of oxygen-17 was observed in the brain extracted from rats that inhaled oxygen-17 gas. The in vivo study demonstrated that there were changes in the ^{1}H-NMR image intensity of brain of rats that inhaled oxygen-17 gas. These facts indicate that oxygen-17 can serve as a tracer in the study of cerebral oxygen utilization.

Oxygen and glucose are the essential substrates of cerebral energy metabolism. Therefore, the determination of local cerebral utilization of these substrates should provide salient information about the relationship between physiologic function and energy metabolism in the central nervous system. The local cerebral glucose utilization has been already investigated extensively using a [^{14}C]deoxyglucose method (1, 2). However, there have been no methods for investigating the local cerebral oxygen utilization in small animals because there has been no adequate radioisotope of oxygen available. A non-radioactive isotope of oxygen, oxygen-17, may be useful as a tracer because it has a magnetic moment and can

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be detected by ^{17}O-NMR spectroscopy. Furthermore, oxygen-17 has a magnetic interaction with proton nuclei (^{1}H) and in the form of $H_2{}^{17}O$ shortens their proton NMR transverse relaxation times (T_2), which produces changes in ^{1}H-NMR image intensity (3, 4). Therefore, the fate of externally administered oxygen-17 to an animal can be traced directly by ^{17}O-NMR spectroscopy and indirectly by an ^{1}H-NMR imaging system, which may be utilized for the investigation of local cerebral oxygen utilization.

In order to verify this proposition, the NMR spectra of oxygen-17 in organs including the brain extracted from small animals (rat) were observed in the presence or absence of oxygen-17 administration. The changes in ^{1}H-NMR image intensity of the brains of rats that inhaled oxygen-17 gas were also investigated.

MATERIAL AND METHODS

Male Wistar rats weighing between 300 and 350 g were used. After the animals were anesthetized with halothane and immobilized with pancuronium, a tracheostomy tube was inserted and connected to a small animal ventilator set to deliver the desired gas.

For the in vitro NMR study, one rat inhalated 40 atom% oxygen-17 gas in total 20% oxygen/balance nitrogen for 7.5 min, and another rat inhaled this mixture for 13.7 min. After the cessation of the inhalation, each rat was rapidly decapitated, and its organs (brain, liver, and blood) were extracted, packed into 10-mm NMR tubes, and kept in an ice box. As a control, another three rats were killed without the inhalation of oxygen-17 gas and their organs were also extracted. The NMR spectra of oxygen-17 in organs were recorded with a Nicolet NT-300 NMR spectrometer at 40.67 MHz in the Fourier transform mode. The temperature of the probe was maintained at 4 C.

For the in vivo NMR imaging study, the brain of one rat was scanned using a General Electric 4.7 Tesla NMR imaging system with a proton resonance frequency of 200.29 MHz, before, during, and after the inhalation of oxygen-17 gas. The time required for obtain the T_2-weighted images was 8.3 min with a TE (time to echo) = 80 msec and a TR (repetition time) = 2000 msec.

RESULTS

The NMR spectra of oxygen-17 in the brains obtained from one of the three control rat and from rats that inhaled oxygen-17 are shown in Figure 1. For the longer time the rat inhaled the oxygen-17 gas, the larger spectrum was obtained. When the signal

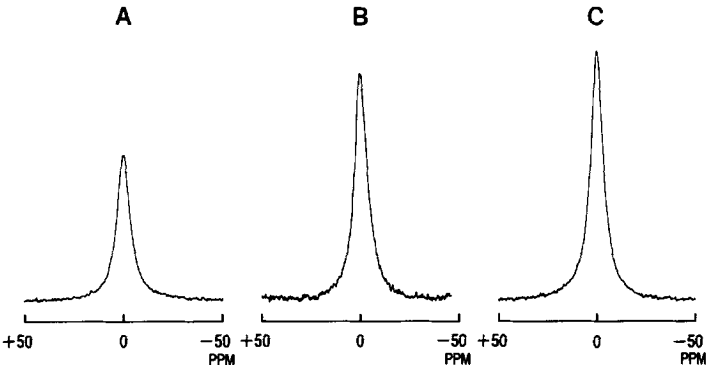

Figure 1. The NMR spectra of oxygen-17 in brain extracted from A) a rat that did not inhale oxygen-17 gas, B) a rat that inhaled the gas for 7.5 min, and C) a rat that inhaled the gas for 13.7 min.

amplitude in a control brain was normalized to 100%, the amplitude in the brains from the rats that inhaled oxygen-17 gas for 7.5 and 13.7 min were 138 and 155%, respectively. On the other hand, there was no significant difference in the signal amplitude among the three control brains extracted from the three control rats. The same tendency was observed in the spectra obtained from the liver and blood (Figure 2).

Figure 3 shows the T_2-weighted images obtained before, during, and after the inhalation of the oxygen-17 gas. Compared to the image obtained before the inhalation (Figure 3A), the image

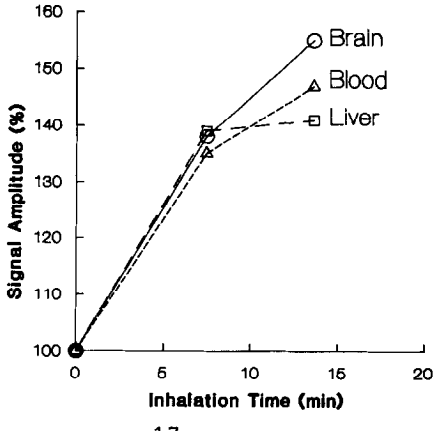

Figure 2. The increment of ^{17}O-NMR signal amplitude in the brain blood, and liver, when the amplitude in each organ extracted from a control rat was normalized to 100%. 'Inhalation Time' shows how long a rat inhaled oxygen-17 gas.

obtained during and after the inhalation exhibited the decrease in ^{1}H-NMR image intensity (Figure 3B, 3C and 3D).

DISCUSSION

In the study using ^{17}O-NMR spectroscopy, spectra consisting of a broad but single line were obtained from all the studied organs (brain, liver, and blood) despite the presence or absence of oxygen-17 administration. These spectra were considered to be resonances of oxygen-17 existing in tissue water ($H_2{}^{17}O$), because other forms of oxygen-17 existing in the organs, such as hemoglobin-bound oxygen-17 and oxygen-17 incorporated into proteins and lipid structures, have inherently poor NMR sensitivity, and the amounts of such compounds are too small to be detected by ^{17}O-NMR spectroscopy. Therefore, the spectra of oxygen-17 in organs extracted from rats that did not inhale oxygen-17 gas reflect the naturally existing $H_2{}^{17}O$ in tissue water (0.037%). This is supported by the fact that there was no difference in signal amplitude among the organs extracted from the three control rats. On the other hand, the increments of signal amplitude in the organs from rats that inhaled oxygen-17 gas show the $H_2{}^{17}O$ newly incorporated into tissue water (metabolic water). The further increments of signal amplitude in the organs from the rat that inhaled more oxygen-17 gas supports this idea. In the study using the ^{1}H-NMR imaging system, the production of oxygen-17 enriched metabolic water from the inhalated oxygen-17 gas is considered to induce the changes in ^{1}H-NMR image intensity.

Coupling with the electron-transport chain in cellular mitochondria, high-energy compounds (ATP) are produced and electrons are finally transferred to oxygen, which results in the production of metabolic water (5). Therefore, the oxygen utilization in a specific organ can be evaluated by quantification

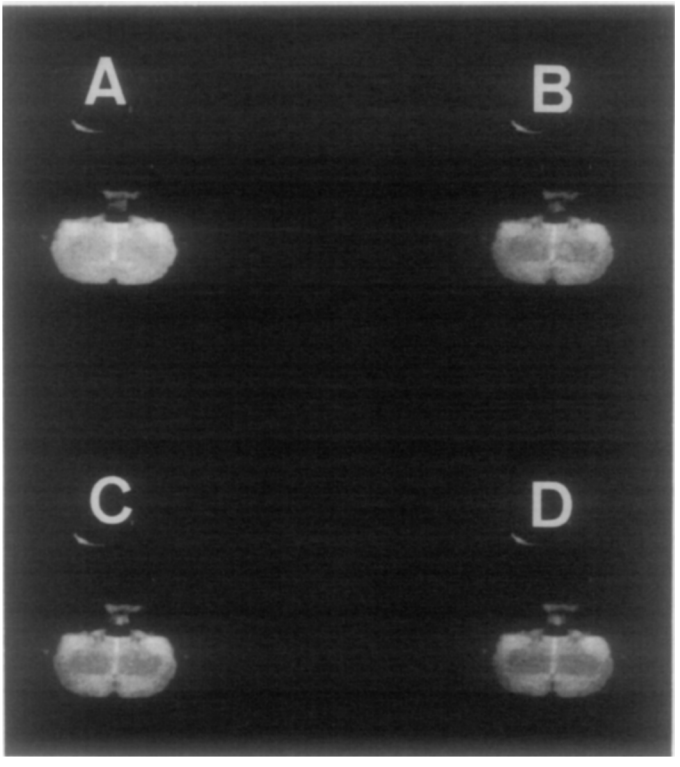

Figure 3. The ^{1}H-NMR images of a rat brain, which were obtained before the inhalation of oxygen-17 gas (A), during (B), 10 min after (C), and 20 min after the inhalation (D).

of the metabolic water produced in the organ. In fact, the measure of regional cerebral oxygen utilization using radioactive oxygen-15 is done on the basis of this theory (6, 7). However, the half-life of oxygen-15 is too short (123 sec) to be available in a laboratory without special facilities such as a cyclotron. In contrast, it is easy to handle the stable isotope oxygen-17. Although another stable isotope of oxygen (oxygen-18) is also available for use as a tracer in mass spectrometer (8), the produced metabolic water ($^{18}H_2O$) cannot be visualized by an ^{1}H-NMR imaging system.

At this point, there are still some problems when we try to determine local cerebral oxygen utilization by means of oxygen-17 and an ^{1}H-NMR imaging system. As shown in Figure 2, the more the

rat inhaled oxygen-17 gas, the larger the observed spectrum, and this was true not only in the brain but also in the liver and blood. This fact indicates that $H_2{}^{17}O$ is produced in various organs and then washed into the blood circulation. Therefore, it is possible that some of changes in ^{1}H-NMR image intensity may be induced by the oxygen-17 enriched metabolic water produced in other organs, which is perfused from the systemic circulation into the brain. In order to distinguish these two types of metabolic water, the time course of the appearance of $H_2{}^{17}O$ in the brain and blood after the administration of oxygen-17 should be examined precisely by ^{17}O-NMR spectroscopy. The optimization of a pulse sequence in a ^{1}H-NMR imaging system for the detection of even small changes in the amount of $H_2{}^{17}O$ will be also required.

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