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Brain magnetic resonance imaging with contrast dependent on blood oxygenation

(cerebral blood flow/brain metabolism/oxygenation)

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ABSTRACT **Paramagnetic deoxyhemoglobin in venous blood is a naturally occurring contrast agent for magnetic resonance imaging (MRI). By accentuating the effects of this agent through the use of gradient-echo techniques in high fields, we demonstrate *in vivo* images of brain microvasculature with image contrast reflecting the blood oxygen level. This blood oxygenation level-dependent (BOLD) contrast follows blood oxygen changes induced by anesthetics, by insulin-induced hypoglycemia, and by inhaled gas mixtures that alter metabolic demand or blood flow. The results suggest that BOLD contrast can be used to provide *in vivo* real-time maps of blood oxygenation in the brain under normal physiological conditions. BOLD contrast adds an additional feature to magnetic resonance imaging and complements other techniques that are attempting to provide positron emission tomography-like measurements related to regional neural activity.**

Magnetic resonance imaging (MRI) is a widely accepted modality for providing anatomical information. Current research (1) involves extending MRI methods to provide information about biological function, in addition to the concomitant anatomical information. In addition to localized spectroscopy (2) and chemical shift imaging (3) that are applicable to many chemical species, MRI of water protons has been functionally extended to NMR angiography (4), perfusion imaging (5, 6), and perfusion imaging enhanced by exogenous contrast agents (7). Since water is by far the predominant molecule in tissue, and since its signal dominates the information content in proton images, one would ideally like to exploit changes in the water signal that arise from physiological events. Except for cases of water movement, such as blood flow, these changes are normally very small.

It has previously been demonstrated (8, 9) that the presence of deoxyhemoglobin in blood changes the proton signal from water molecules surrounding a blood vessel in gradient-echo MRI, producing blood oxygenation level-dependent (BOLD) contrast. BOLD contrast has its origin in the fact that when normally diamagnetic oxyhemoglobin gives up its oxygen, the resulting deoxyhemoglobin is paramagnetic. The presence of paramagnetic molecules in blood produces a difference in magnetic susceptibility between the blood vessel and the surrounding tissue. This susceptibility difference is "felt" both by the water molecules in the blood and by those in the surrounding tissue, the effect extending significantly beyond the vessel wall. This increase in the number of spins affected by deoxyhemoglobin is a form of amplification. When the susceptibility-induced local field differences exist within an imaging voxel, there is a resultant distribution of shifts in water resonance frequencies. In the gradient-echo method, a phase dispersion of water proton signals is produced at the echo time. This dispersion reduces the signal intensity and the voxel appears dark in the image. These intensity losses, which at high magnetic fields ($\geq$4 T) extend significantly beyond the boundary of the blood vessel, are the source of BOLD contrast. This form of contrast is not observed in spin-echo images. Through simulations (9), we have shown that vessels as small as 50 μm in diameter can be detected in images with a pixel size of 100 μm. We have also demonstrated that the size of the susceptibility-induced local field depends on (*i*) the concentration of paramagnetic deoxyhemoglobin and (*ii*) the orientation of the vessel relative to the main magnetic field (8, 9).

Since BOLD contrast depends on the state of blood oxygenation, physiological events that change the oxy/deoxyhemoglobin ratio should lend themselves to noninvasive detection through the accentuation of BOLD contrast in gradient-echo proton images at high magnetic fields. We report here that this is indeed the case and demonstrate changes in BOLD-contrast microimages of brain produced by changes in inhaled gas mixture under urethane anesthesia, by insulin-induced hypoglycemia under diazepam sedation, and by changes in the level of halothane anesthesia. The observed changes in BOLD contrast correlate with the anticipated changes in blood oxygen level produced by altered metabolic load or blood flow (10).

MATERIALS AND METHODS

Physiology and Anesthetics. Sprague–Dawley rats (female, 250 $\pm$ 25 g) were anesthetized with the described anesthetics and ventilated without assistance. Animal care and the administration of anesthetics were in accordance with National Institutes of Health guidelines. For MRI experiments, the animal was placed in a thermally regulated compartment within the magnet and rectal temperature and electrocardiogram were monitored. Blood was periodically sampled from the femoral artery and blood gas concentrations were measured with a Radiometer model ABL330 gas analyzer. Blood glucose was estimated using a Boehringer–Mannheim Accu-Check IIm. To measure the electroencephalogram (EEG) during MRI experiments, small tubular salt bridges (0.8 mm i.d.) were implanted in small holes through the skull that were located inside the area encircled by the surface coil used for NMR signal detection. Care was taken not to break the dura.

Magnetic Resonance Microimaging. Gradient-echo images were obtained with a 7-T horizontal magnet at 300 MHz (Spectroscopy Imaging Systems, Fremont, CA). Surface coils of 20 to 25-mm diameter were positioned on the surface of the cranium and used for both excitation and detection of signals. The maximum field gradient used to produce images was 4 G/cm (1 G = 0.1 mT). The slice width of the images shown was 500 μm and the in-plane pixel size was 117 μm $\times$

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Abbreviations: MRI, magnetic resonance imaging; BOLD, blood oxygenation level-dependent; EEG, electroencephalogram.

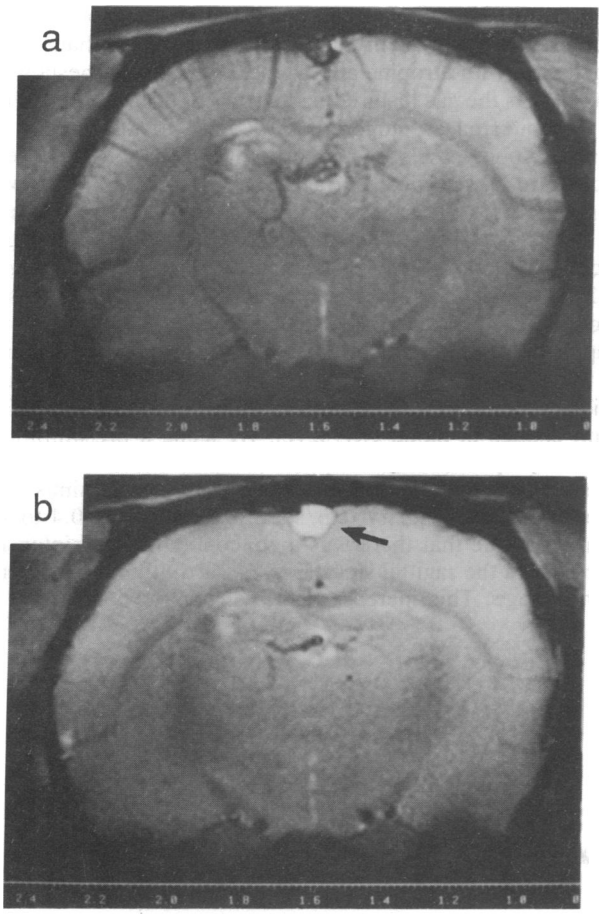

FIG. 1. Effect of blood CO_2 level on BOLD contrast. (*a*) Coronal slice brain image showing BOLD contrast from a rat anesthetized with urethane. The gas inspired was 100% O_2. (*b*) The same brain but with 90% O_2/10% CO_2 as the gas inspired. BOLD contrast is greatly reduced. The arrow points to the sagittal sinus, showing increased signal intensity, indicative of increased blood oxygenation. The magnetic field was perpendicular to the slice plane. The imaging time was 9 min with a repetition time of 0.26 sec and an echo time of 12 msec. The pixel size of the images was 117 μm × 117 μm with a slice thickness of 550 μm. The scale represents linear dimension in cm. Core body temperature was maintained at 35–36.5°C.

117 μm. The echo time of the imaging was 12–18 msec and the image acquisition time was 6–15 min.

Measurement of Blood Flow Velocity and Effect of Blood Oxygenation on Signal Intensity at the Sagittal Sinus. The venous blood flow velocity (cm/sec) at the sagittal sinus was measured by the method of presaturation recovery by flow (11). Proton spins within a 1-mm- or 2-mm-thick slice were selectively presaturated and after a specified delay time a 0.5-mm-thick slice at the same location was selected for image observation by a gradient-echo pulse sequence with 12-msec echo time. The major direction of the blood flow in the sagittal sinus was normal to the slice plane. The venous blood signal recovered with increasing delay time, providing a measure of blood flow velocity. In this presaturation-recovery-by-flow method, the contribution to the flow signal by the venous blood drainage into the sagittal sinus within the presaturation slice was minimized because of the slow longitudinal relaxation time (T_1) recovery of these spins.

In the standard gradient-echo images shown in the figures (no presaturation), the signal intensity of the venous blood water at the sagittal sinus depends on the blood oxygenation. This is because the transverse relaxation time (T_2) of blood water is strongly dependent on blood oxygenation (12). At the 7-T field strength used in this study, T_2 varies from 50 msec

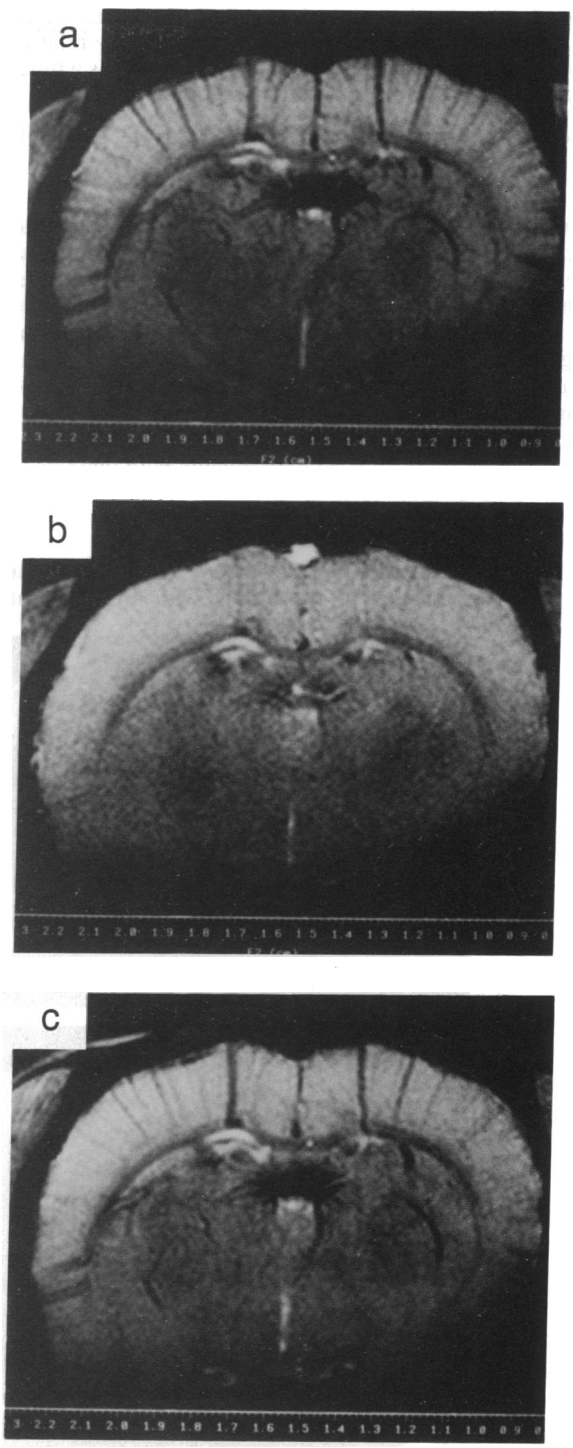

FIG. 2. Effect of insulin-induced hypoglycemia on BOLD contrast. (*a*) After injection of bovine insulin, BOLD contrast is apparent at blood glucose levels ≥20 mg/dl. (*b*) Loss of contrast is observed at blood glucose levels << 20 mg/dl. (*c*) BOLD contrast can be restored by intraperitoneal injection of mannose. Inhalant gas, 50% O_2/50% N_2; core body temperature was maintained at 34–35°C;

at 100% oxygenation to 4 msec at 0% oxygenation. The venous blood signal becomes very weak when the T_2 value becomes comparable to or shorter than the echo time of the signal acquisition. At 60% oxygenation level, the estimated T_2 value is 18 msec, similar to the echo time used in this report.

Simulation of BOLD-Contrast Images. The image simulation (see ref. 9) assumed a cylinder of radius *a* oriented

perpendicular to the applied magnetic field and having a volume magnetic susceptibility difference of $\Delta\chi$ relative to the surrounding tissue. The frequency shift (ω_s) as a function of position used in the simulation was then

$$\text{outside the cylinder: } \omega_s/\omega_0 = 2\pi\Delta\chi(r/a)^2[(2\cos^2\theta - 1)]$$

$$\text{inside the cylinder: } \omega_s/\omega_0 = -(2\pi/3)\Delta\chi,$$

where $\mathbf{r}$ (r, θ) is the position vector relative to the center of the cylinder with angle θ to the magnetic field. The resonance frequency without the local field is ω_0. The field inside the cylinder was assumed to be homogeneous, but the T_2 decay rate of blood water had a quadratic dependence on the oxygenation level (12). The voxel size in the simulation was 100 μm × 100 μm × 500 μm and the pixel size in frequency along the read gradient was 100 Hz. The maximum frequency shift at the surface of the vessel ($2\pi\omega_0\Delta\chi$) was 200 Hz, similar to that measured in *in vitro* experiments with deoxygenated blood (9). The echo time was 10 msec.

RESULTS

Effects of Anesthetics and Blood Flow on BOLD Contrast. Coronal slice images of rat brains with BOLD contrast demonstrating that image contrast is reduced as blood flow is increased are shown in Fig. 1. Rats under urethane (850 mg/kg i.p. with atropine at 0.04 mg/kg s.c.) anesthesia with 100% O_2 as the gas inspired had strong BOLD contrast (Fig. 1*a*), manifested as numerous dark lines in the vicinity of venous blood vessels. In neocortex these vessels lie perpendicular to the cortical surface. This BOLD contrast was completely lost (Fig. 1*b*) when the gas inhaled was switched to 10% CO_2/90% O_2. The addition of CO_2 to the inhalant gas increased blood CO_2 levels from 50 to 80 mmHg (1 mmHg = 133 kPa). In general, increased blood CO_2 produces increased blood flow. This increased blood flow provides a greater supply of oxygen to the brain and, in the absence of a change in the metabolic load, should increase venous blood oxygenation, consistent with our observed loss of BOLD contrast with change in blood CO_2 level. By using a presaturation-recovery-by-flow MRI method, we could directly measure the increase in blood flow produced in the sagittal sinus. The measured flow velocity (cm/sec) increased from 0.4 to 1.7 cm/sec. Note that there was a concomitant signal intensity increase at the sagittal sinus (Fig. 1*b*, arrow) in the gradient-echo image. This increase also indicates a higher level of

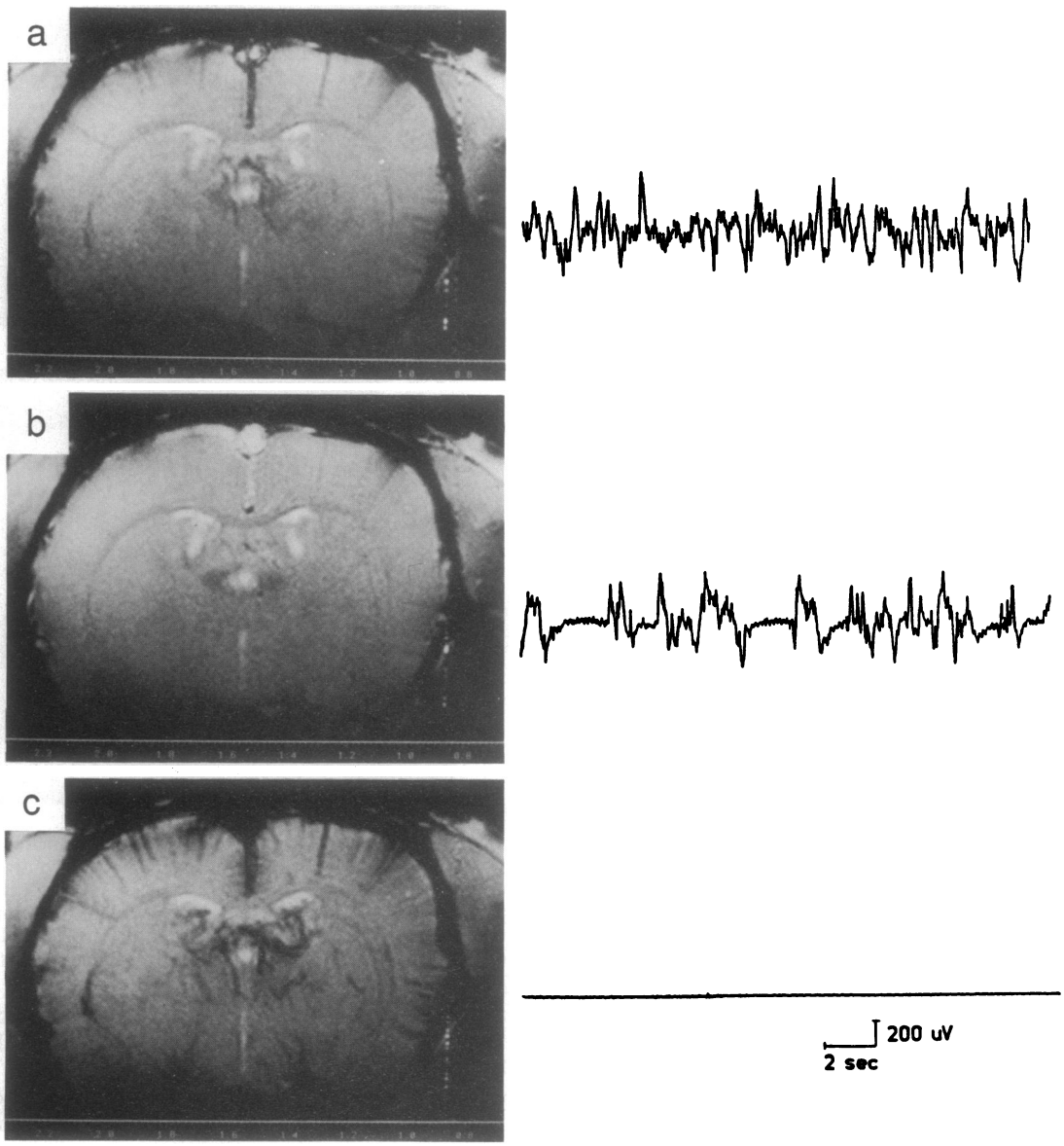

FIG. 3. Dependence of BOLD image contrast halothane dose. Brain images of a rat inspiring 0.75% halothane in oxygen (*a*), 3% halothane in oxygen (*b*), or 100% N_2 (*c*) are shown. Also shown are EEG measurements at each halothane level shortly before the images were acquired.

oxygenation in the venous blood, because of the known dependence of water T_2 on blood oxygenation. Thus the loss of BOLD contrast and the high venous blood oxygenation level measured at the sagittal sinus were consistent with the large increase in blood flow velocity induced by the increased systemic CO_2.

Insulin-Induced Hypoglycemia Reduces BOLD Contrast. As shown in Fig. 2, under diazepam sedation (10 mg/kg) and with 50% O_2/50% N_2 as the inhalant gas, a rat brain image demonstrated a BOLD contrast slightly more pronounced than that in Fig. 1*a*. (The signal intensity at the sagittal sinus was less than that in Fig. 1*a*, suggesting a lower level of blood oxygenation under these conditions.) After injection of insulin (bovine, 16 units/kg; Sigma), BOLD contrast did not change while blood glucose remained >20 mg/dl (Fig. 2*a*). When blood glucose dropped to well below 20 mg/dl, BOLD contrast was greatly reduced (Fig. 2*b*). Addition of glucose or mannose (400 mg/kg i.p.) reversed this change, restoring the contrast (Fig. 2*c*).

The reduction in BOLD contrast during hypoglycemia was correlated with a modest increase in blood velocity at the sagittal sinus. $Paco_2$ levels in blood before insulin-induced hypoglycemia were 35–40 mmHg with blood velocity at the sagittal sinus of 0.4–0.6 cm/sec. When the loss of BOLD contrast occurred, $Paco_2$ was 30–38 mmHg and blood velocity was 0.7–1.1 cm/sec. However, it seems likely that the loss of BOLD contrast produced by strong hypoglycemia was not entirely produced by the increase in blood flow but also from a reduced metabolism, since, at normal glucose levels, modest increases in blood flow produced by other methods did not cause a complete elimination of BOLD contrast (data not shown).

BOLD-Contrast Reduction Without Increased Blood Flow. When the inhalation anesthetic halothane is used, it is possible to vary the state of anesthesia of the animal by varying the concentration of halothane and to demonstrate a change in BOLD contrast not produced by changes in blood flow velocity. We have compared a series of brain images from the same animal produced under identical imaging but different degrees of anesthesia. To obtain an estimate of overall neuronal activity, the EEG signal was measured *in situ* before or after image acquisition from electrodes positioned on the dura near the imaged coronal section. The dose dependence of the BOLD contrast is shown in Fig. 3, along with the corresponding EEG signals. With 0.75% halothane in oxygen as the inhalant gas, radiating venous blood vessels were observed in the neocortex, demonstrating BOLD contrast (Fig. 3*a*). Increasing the halothane content to 3% led to depression of the EEG (brief periods of isoelectric signal are evident) and concomitant loss of contrast in the MRI image (Fig. 3*b*). Finally, to exhibit maximum possible contrast, the anesthetized animal was ventilated with 100% nitrogen, leading to an isoelectric EEG. The highly deoxygenated blood in the brain under this condition gave very high BOLD image contrast (Fig. 3*c*). Unlike the urethane experiments described above, it is unlikely that the decreased image contrast observed with increasing halothane content can be explained as an effect of halothane on cerebral blood flow or volume. In separate experiments the venous blood flow velocity was measured at various halothane levels. Flow velocity actually decreased from 0.25 cm/sec at 0.75% halothane to 0.12 cm/sec at 3% halothane. The decreased BOLD contrast in Fig. 3*b* is therefore probably due to the lowered oxygen consumption at 3% halothane, where the animal reached a state of low brain activity as judged by the EEG.

Image Simulation. As a step toward quantifying expected changes in BOLD contrast, we have used image simulation to estimate its dependence on blood oxygenation. Image simulations were performed for a cylindrical blood vessel perpendicular to the main magnetic field, using experimentally determined values for the magnetic parameters of the blood and the surrounding tissue. The relation between BOLD contrast and blood oxygenation that resulted from the simulations is shown in Fig. 4*a* for a variety of blood vessel sizes. The normalized BOLD contrast, $(I_o - I)/I_o$, changed in the range of blood oxygenation between 90% and 50%, the anticipated range of venous blood oxygenation. The apparent width of the vessel in the image is also blood oxygenation dependent for those with diameters that exceed the pixel size (9). An anoxic brain image is shown with a corresponding signal intensity profile in Fig. 4*b*. An evaluation of the sensitivity of BOLD contrast to the change in the oxygen tension in the blood can be made for this image from the simulation results. If the minimum detectable change in the contrast/noise ratio ($\Delta I/N$) is assumed to be 2 in an image with a signal/noise ratio (I_o/N) of 40, the corresponding change in the blood oxygenation level (Y) at $Y \approx 0.5$ in a 50-μm size vessel would be 0.15. Taking account of the cooperative oxygen binding to hemoglobin in blood, this change in Y relates to a change of 20% in the blood oxygen tension. The sensitivity of the method will be reduced for vessels that are not orthogonal to the magnetic field direction. In this regard, the vasculature of the cortex is advantageous as it runs predominantly in the coronal plane.

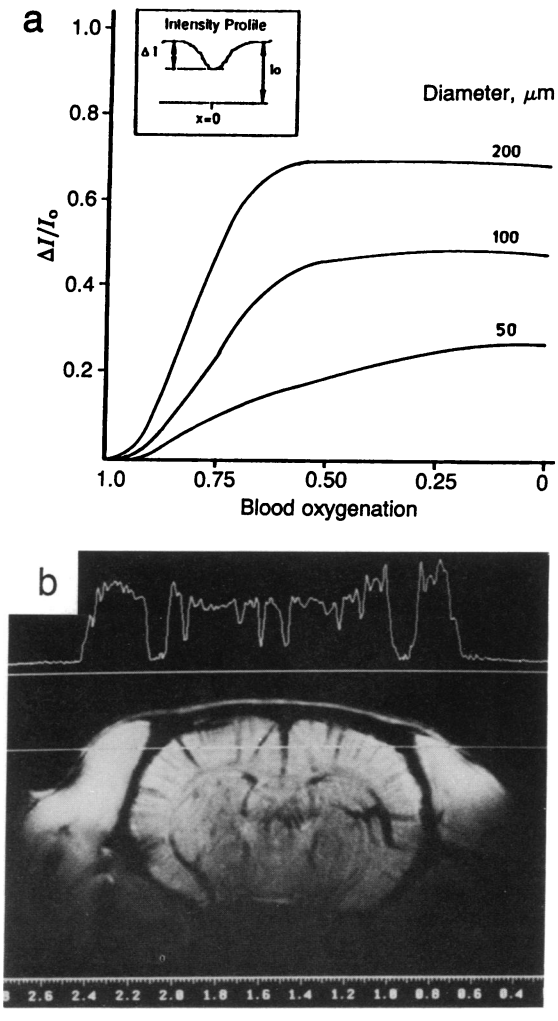

FIG. 4. (*a*) Relationship between BOLD contrast and blood oxygenation. The image simulation was performed for a blood vessel with its longitudinal axis perpendicular to the magnetic field and also perpendicular to the read-gradient axis in the slice plane. (*b*) Signal intensity profile along the line shown in the accompanying image of an anoxic brain (ventilation with 100% N_2).

In order to use BOLD contrast to quantitatively determine regional venous blood oxygenation, *in vivo* calibration will be necessary. For example, images obtained at two different average blood oxygenation levels, produced by different O_2 compositions in the inhalant gas, provide sufficient information so that local contrast changes in subsequent images of the same area can be related to changes in local blood oxygenation quantitatively.

DISCUSSION

We have demonstrated that BOLD-contrast MRI of brain can track the changes in blood oxygenation expected from anesthetic inhaled gases and from insulin-induced hypoglycemia that change the physiological state of the animal. Since the contrast is entirely dependent on blood oxygenation, it is determined by the balance of supply (blood flow) and demand (extraction by tissue) of oxygen. Under normoxic conditions arterial blood is fully oxygenated and does not contribute to BOLD contrast, while venous blood vessels containing deoxygenated blood contribute dark lines to the image. The results shown here indicate that BOLD contrast can be used to noninvasively monitor in real time the blood oxygenation levels of brain areas in response to central nervous system drugs that affect basal metabolism or blood flow. Although BOLD-image contrast is enhanced at high magnetic fields, the effect is observed at 4.7 T*, a field strength that is close to the highest field strength (4 T) presently available for human subjects.

Many MRI methods are being explored as alternative approaches to positron emission tomography (PET) in the study of regional brain activity, including methods that measure regional blood flow and volume using exogenous contrast agents (7). PET imaging relies on a family of tracer methods for measuring different physiological quantities including blood volume, blood flow, and regional oxygen extraction (13). BOLD contrast adds to a similar, emerging set of functional MRI methodologies that are likely to be complementary to PET imaging in the study of regional brain activity. Unlike most tracer methods, BOLD contrast relies on an intrinsic contrast agent and image acquisition can be precisely synchronized to external stimuli with good time resolution. Even transient changes in blood oxygenation (14) can, in principle, be measured.

The underlying mechanism of BOLD contrast is the accentuation by gradient-echo imaging at high magnetic fields of effects produced by magnetic susceptibility variation that is caused by an endogenous paramagnetic agent. The same mechanism may also be useful in the study of other iron-containing systems, including the spleen, liver, and bone marrow.

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