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Systemic Theophylline Augments the Blood Oxygen Level–Dependent Response to Forepaw Stimulation in Rats

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BACKGROUND AND PURPOSE: **Functional MR imaging with blood oxygen level–dependent (BOLD) contrast enhancement is believed to rely on changes in cerebral blood flow and deoxyhemoglobin level to estimate the location and degree of neural activation. We studied the relationship between neural activation and the observed BOLD response by using theophylline, an antagonist of the inhibitory neurotransmitter adenosine and a potent inhibitor of the vasodilatory response to neural activation.**

METHODS: **Using a rat model with electrical forepaw stimulation, we performed fMRI measurements before and after the systemic injection of either theophylline (0.1 mmol/kg) or an equivalent volume of saline. Changes in the BOLD response were quantified by determining the number of activated voxels and the amplitude of the BOLD response for each animal in the theophylline and saline groups.**

RESULTS: **The theophylline group had a significantly increased BOLD response (70–150% increased activated voxel count and 60–65% increased BOLD response amplitude) at 45 and 60 minutes after systemic injection compared with baseline. The response of the saline-injected control group did not change significantly.**

CONCLUSION: **The administration of systemic theophylline significantly augmented the BOLD response due to either an elevation of resting deoxyhemoglobin levels or the neuroexcitatory effect of theophylline. This effect potentially could be used in human fMRI studies to increase the sensitivity of the BOLD response.**

Functional MR imaging (fMRI) with blood oxygen level–dependent (BOLD) contrast enhancement is believed to reflect the relative levels of oxy- and deoxyhemoglobin in the brain, with the local balance changing as nearby neural activity increases. The relationship is complex, however, because the levels of oxyhemoglobin and deoxyhemoglobin depend on the local oxygen consumption, as well as the local cerebral blood flow response to neural activity. Findings from optical studies (1) support the hypothesis that an activation-induced increase in local cerebral blood flow causes a dilutional decrease in deoxyhemoglobin levels. These near infrared spectrometry and laser Doppler results show a clear increase in localized cerebral blood flow and a decrease in the local concentration of deoxyhemoglobin in the region of activated cortex. Changes in the deoxyhemoglobin concentration are believed to be the primary cause of BOLD contrast (2, 3).

On the basis of the model of BOLD theory, one can hypothesize that a change in the vascular response to neural activation alters the pattern of BOLD activation. The purpose of this study was to test this model by using theophylline, a compound known to perturb the activity of adenosine, a putative activation-flow coupling agent (4). Theophylline, an antagonist of adenosine A-1 and A-2 receptors, mediates its effect by crossing the blood-brain barrier and blocking adenosine A-2 receptors on perivascular smooth muscle cells (5). Direct visual observations made by using a closed-cranial-window rat preparation have shown that 0.05–0.2 mmol/kg of systemic theophylline significantly attenuates the vasodilatory response to neural activation (6–8); it also produces a mild baseline decrease in cerebral blood flow (6).

Using a rat forepaw stimulation model, we sought to determine the effect of systemic theophylline on

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Physiologic parameters in animals undergoing BOLD experiments

Group	pH	P_{CO_2} (mm Hg)	Pa_{O_2} (mm Hg)	MABP (mm Hg)*
Systemic saline, control animals (n = 4)				
Preinjection	7.42 ± 0.03	36 ± 1.6	152 ± 29	100 ± 8
Postinjection	7.41 ± 0.04	37 ± 4.9	126 ± 38	96 ± 12
Systemic theophylline, test animals (n = 5)				
Preinjection	7.40 ± 0.04	36 ± 1.9	157 ± 27	106 ± 7
Postinjection	7.38 ± 0.02	37 ± 2.1	166 ± 11	106 ± 10

Note.—Postinjection values were recorded approximately 90 minutes after systemic injection. Pre- and post-injection values of the parameters did not differ significantly in either the test group or control group ($P = 0.5$, t test). The groups did not differ significantly ($P = .05$, t test).

* MABP indicates mean arterial blood pressure.

the BOLD response, as well as the time course of this effect.

Methods

Animal Preparation

The University of Washington Animal Care Committee approved all animal procedures. Our preparation was based on a well-characterized rat model of cerebrovascular autoregulation (9). It involved a forepaw stimulation paradigm similar to that of Hyder et al (10), and it has been previously described (11). Briefly, adult, male 350–450 g Sprague-Dawley rats (B & K, Kent, WA) were anesthetized with 4% halothane (Halocarbon Laboratories, River Edge, NJ) and then maintained with 1–2% Halothane and 100% O_2. Surgical incision sites were further anesthetized with subcutaneous injections of 2% mepivacaine hydrochloride (Astra USA, Westborough, MA). Then, a femoral artery and vein were catheterized by using 24- and 22-gauge catheters, respectively (AngioCatheter; BD Instruments, Franklin Lakes, NJ), and continuous monitoring of arterial blood pressure and heart rate was initiated by using an MR-compatible monitor (BPA-2000 analyzer; Micro-Med, Louisville, KY). A 22-gauge intraperitoneal catheter was then placed through a small, anterior midline abdominal incision. Next, tiny, subcutaneous needle electrodes (made from 30-gauge copper transformer wire) were subcutaneously placed in the radial and ulnar aspects of the left forepaw. Finally, tracheotomy was performed, and the trachea was cannulated with a 14-gauge catheter. Immediately after the initiation of mechanical ventilation, the animal was paralyzed with D-tubocurarine chloride (Abbott Laboratories, North Chicago, IL; 1 mg/kg intravenous administration).

Next, a 1:10 (by weight) mixture of α-chloralose (Sigma, St Louis, MO; 40 mg/kg) and urethane (Sigma; 400 mg/kg) was administered intraperitoneally (IP), and the halothane anesthesia was gradually discontinued during the ensuing 15 minutes. The inspired gas was then changed to a mixture of oxygen and room air; the resultant FiO_2 was 60%. Finally, the animal was placed in an MR-compatible stereotactic head holder. MR imaging was delayed until at least 1 hour after the discontinuation of halothane anesthesia to ensure adequate washout.

On the basis of the results of the arterial blood gas analysis, ventilator settings and the FiO_2 were adjusted to maintain the following parameters: pH, 7.35–7.45; P_{CO_2}, 35–45 mm Hg; and Pa_{O_2}, 110–180 mm Hg (Table). The animal's body temperature was maintained by using a circulating water warming blanket (Gaymar Industries, Orchard Park, NY). Supplemental doses of D-tubocurarine chloride (1 mg/kg) were administered every 30 minutes for the duration of the experiment. Supplemental doses of α-chloralose (10–15 mg/kg IP) with urethane (100–150 mg/kg IP) were administered approximately every 60 minutes for the duration of the experiment.

Once gradient shimming, radio-frequency (RF) transmitter, and RF receiver gains were set, these parameters and the animal's position within the magnet remained unchanged for the duration of the experiment. At the end of each experiment, the rat was euthanized under anesthesia, according to approved protocol.

BOLD fMRI

All experiments were performed with a 1.5-T Horizon Echo Speed whole-body MR machine (GE Medical Systems, Milwaukee, WI) in an RF-shielded room. A 2.5-cm transmit-receive surface coil (designed in our laboratory by Cecil Hayes, PhD) was used for all measurements.

We used a standard multisection, two-dimensional, single-shot, gradient-echo, echo-planar pulse sequence for all functional imaging examinations. BOLD fMRI pulse sequence parameters were as follows: TR/TE/NEX, 2000/50/1; flip angle, 90°, FOV, 7 × 4 cm; matrix, 70 × 40; and section thickness, 1 mm. The frequency axis and the 7-cm FOV axis were oriented left-right. Both fat saturation and ramp sampling were enabled. These parameters resulted in 1-mm^3 voxels. Ten section locations were imaged at each time point, with 51 image sets in each BOLD fMRI measurement. Each measurement lasted 102 seconds and generated 510 images. fMRI measurements were obtained at intervals of approximately 15 minutes.

Forepaw electrical stimulation was accomplished with 5-V, 0.3-ms, 3-Hz pulses generated by a pulse generator (Grass Instruments, Quincy, MA). The maximal current delivered during each pulse was 0.75–1.0 mA. A boxcar pattern of forepaw stimulation was used (off/on/off/on/off for 11/10/10/10/10 image sets). The pulse generator was controlled by a computer (PowerMac 8100/100; Apple Computers, Cupertino, CA) running PsyScope software (12) via a hardware interface (PsyScope Button Box; New Micros, Dallas, TX). A monostable multivibrator (74123; Texas Instruments, Dallas, TX) was used to shape the scope trigger output from the integrated pulse generator module of the MR unit. This setup allowed PsyScope to count each image in the fMRI acquisition as it was obtained and use this count to enable and disable the pulse generator at precise predetermined times during the acquisition. We ensured that the forepaw stimulation pulses did not interfere with the MR measurements by using bipolar forepaw electrodes constructed from twisted-pair insulated copper wire to minimize RF emissions.

Theophylline Effect on the BOLD Response

Animals were divided into two groups. The test group (five animals) received theophylline, 0.1 mmol/kg IP, which was dissolved in 1 mL normal saline. The control group (four animals) received 1 mL normal saline IP. Five fMRI images were obtained in each animal: one baseline and four postinjection images. Baseline images were obtained 5 minutes before the IP injection. Postinjection images were obtained 15, 30, 45, and 60 minutes after the IP injection. Because of technical problems, one animal in the theophylline group did not undergo 45-minute postinjection imaging.

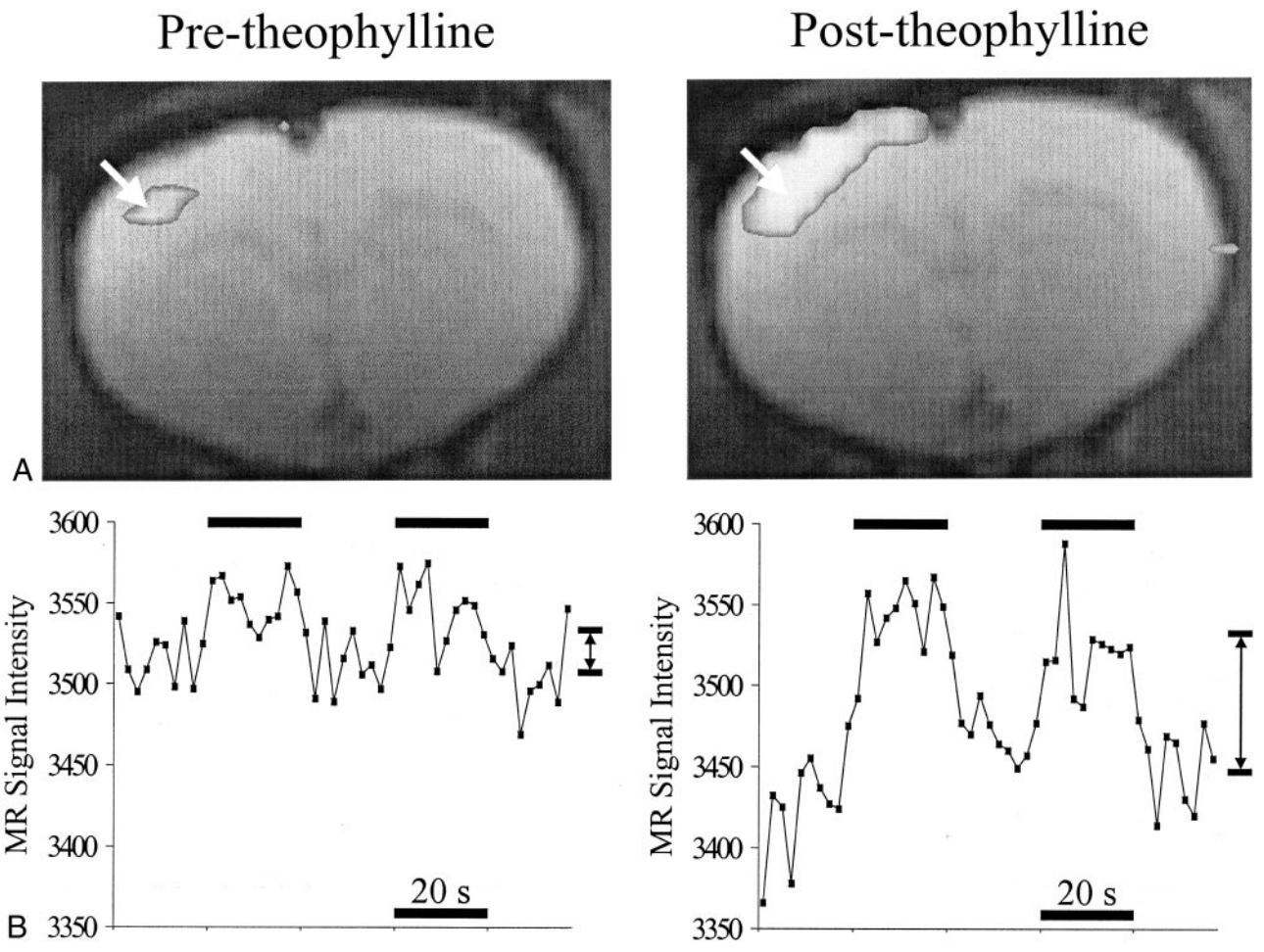

FIG 1. Theophylline effect on the BOLD fMRI response to left forepaw electrical stimulation. Data are shown for a single section through the sensory cortex in a single animal at baseline (*left*) and 60 minutes after the administration of 0.1 mmol/kg systemic theophylline (*right*).

A, The number of activated voxels significantly increased 60 minutes after the administration of systemic theophylline. *Arrows* indicate the most significantly activated voxel, which had the same location on both images.

B, BOLD response for the voxel indicated in *A*. *Bars* to the right of each graph indicate the average signal intensity during forepaw stimulation (*upper bars*) and at rest (*lower bars*). The *double-arrowhead vertical lines* to the right of each graph indicate the BOLD response amplitude. The BOLD response amplitude significantly increased 60 minutes after systemic theophylline compared with the baseline. *Horizontal bars* above each graph indicate periods of forepaw electrical stimulation with a 4-second hemodynamic delay.

Data Analysis

To qualitatively detect areas of BOLD activation, preliminary analysis was performed during the experiment (FuncTools; GE Medical Systems) on a UNIX-based workstation. Images were then extracted into Digital Imaging and Communications in Medicine format (xginx; GE Medical Systems). Detailed quantitative analysis was then performed by using the extracted images and MEDx (Sensor Systems, Sterling, VA) running on a LINUX-based computer. *Z* scores were generated for each voxel by using an unpaired *t*-test statistic referenced to a boxcar waveform that matched the pattern of forepaw stimulation, with a 4-second hemodynamic delay. Activation maps were generated by using thresholds with these *Z*-score maps. The activation threshold was individually determined for each animal, because of variations in overall signal-to-noise ratio between animals. Once determined, the same threshold value was used in every measurement for that animal. Cluster analysis was used to identify clusters of activated voxels.

We observed a single well-defined cluster of activated voxels in each animal in the contralateral S_1 cortical area during each BOLD measurement (Fig 1A). The activated voxel count was defined as the number of active voxels in this cluster. This parameter was reported in normalized form by dividing the activated voxel count from each BOLD measurement by that animal's baseline (ie, pretheophylline or presaline) value.

The most significantly active voxel (ie, that with the highest *Z* score) in the cluster of active voxels was identified in each BOLD measurement (Fig 1A). We found that the location of this voxel remained constant for all BOLD measurements in a given animal. By using data from the most significantly activated voxel, the BOLD response amplitude was defined as the difference between the average signal in this voxel during forepaw stimulation and at rest (Fig 1B). This parameter was reported in normalized form by dividing the BOLD response amplitude for each fMRI measurement by that animal's baseline (ie, pretheophylline or presaline) value.

Statistical Analysis

A two-tailed Student *t* test was used to determine the significance of changes in the BOLD response amplitude and activated voxel count relative to baseline values in the control and theophylline groups.

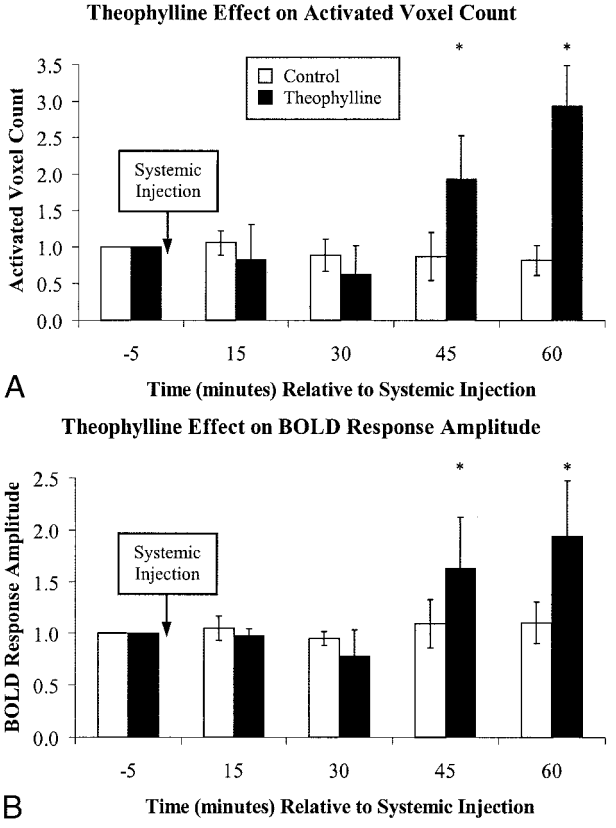

FIG 2. Theophylline effect on the BOLD fMRI response to forepaw electrical stimulation. Results are the average for five test and four control animals. Five measurements were obtained in each animal: one before and four after the systemic injection of either 0.1 mmol/kg theophylline (n = 5) or an equivalent volume of normal saline (n = 4). The timing of each acquisition is relative to the systemic injection. The data for each animal were normalized to the preinjection value. * indicates a statistically significant difference compared with the baseline ($P < .05$).

A, The activated voxel count significantly increased 45 and 60 minutes after the administration of theophylline. It did not significantly change in the control group. The *arrow* indicates when theophylline or saline was administered, just after the first fMRI measurement.

B, The BOLD response amplitude significantly increased 45 and 60 minutes after the administration of theophylline, compared with baseline. It did not significantly change in the control group. The *marker* indicates when IP theophylline or saline was administered, just after the first fMRI measurement.

Results

In both the theophylline group (five animals) and the control group (four animals), a focal region of BOLD activation was centered 1–2 mm anterior to the bregma and 4 mm lateral to midline in the cortex contralateral to the stimulated forepaw. This finding was consistent with the location of forepaw sensory cortex reported in other studies (10, 11, 13–15).

The theophylline group had a significantly increased activated voxel count and BOLD response amplitude at 45 and 60 minutes after systemic administration, compared with baseline (Figs 1, 2). The activated voxel count increased by 70% and 150% at 45 and 60 minutes, respectively, after systemic theophylline administration. Similarly, the BOLD response amplitude increased by 60% and 65% at 45 and 60 minutes, respectively, after systemic theophylline administration. In the control group, these values did not significantly change at any time point after the injection of saline.

Measured physiologic parameters, including the Pa_{O_2}, Pa_{CO_2}, and blood pressure did not significantly change in either group, and no significant differences between the groups were found ($P = .05$, t test) (Table). During forepaw stimulation, the mean arterial blood pressure never changed by more than 2 mm Hg from its value immediately before stimulation.

Discussion

In an attempt to better understand the processes underlying the BOLD response, we studied changes in the BOLD response after perturbation of the normal cerebral blood flow response by using systemic theophylline. Theophylline is a methylxanthine derivative that inhibits cerebral vasodilation by antagonizing adenosine, the putative activation–flow coupling agent (6). Theophylline has been shown to decrease activation-induced vasodilation (6, 7, 16, 17); it also mildly decreases the resting cerebral blood flow.

Our experimental results showed a marked increase in both the BOLD response amplitude and the total volume of tissue in which the activation threshold was reached (as measured by number of activated voxels), compared with pretheophylline measures and findings in saline-treated controls. This increase was observed in all theophylline-treated animals at 45 and 60 minutes after the systemic injection. The time course of this effect was consistent with the measured time course of theophylline levels in the cerebrospinal fluid after IP administration (6).

fMR imaging of the brain relies on a small but measurable increase in regional brain signal intensity of 1–5% that approximates the area of brain activation. This change in signal intensity is believed to be the result of changes in local blood concentrations of oxyhemoglobin and deoxyhemoglobin. Oxyhemoglobin is diamagnetic and has little influence on blood signal intensity. Deoxyhemoglobin, on the other hand, is paramagnetic and causes intravoxel dephasing and a loss of signal intensity. According to the leading theoretical model of BOLD imaging, a steady state level of oxyhemoglobin to deoxyhemoglobin concentrations occurs during resting brain perfusion. After neuronal activation, oxygen utilization increases in the activated tissue; this change is associated with an increase in the regional cerebral blood flow (rCBF) to meet this increased oxygen demand. However, because of the known effects of decoupling between metabolic demand and rCBF, the increase in blood flow is disproportionately large for the modest increase in the amount of oxygen required. The effect of this excess increase in rCBF is the dilution of deoxyhemoglobin in the regional draining venous channels. This relative decrease in the paramagnetic deoxyhemoglobin level results in an increased signal intensity (caused by a decrease in the amount of paramagnetic-related signal loss), compared with the signal intensity in the steady state. The precise

mechanisms that control the changes in rCBF in response to neural activation are incompletely understood.

Other systemic agents have been shown to increase the BOLD response. Several groups report that acute hypercarbia, which causes vasodilation and increased cerebral blood flow, increases the BOLD response (18–21), likely by means of an adenosine-mediated mechanism (22, 23). Acetazolamide, another vasodilator, also increases the BOLD response (21, 24, 25). Theophylline is a generalized vasoconstrictor. With the dose used in this study, theophylline also has an effect of decreasing the vasodilatory response to neural activation (6, 7); in this way, theophylline differs from these other systemic agents. The precise mechanism underlying the effect of each agent on the BOLD response is not well understood. However, given the very different effect of theophylline on the cerebrovascular response to neural activation, one would expect the mechanism by which theophylline augments the BOLD response is different from that of hypercarbia or acetazolamide.

The amplitude of the BOLD response directly depends on the difference between the MR signal intensity during activation and the signal intensity at rest. Increasing this difference increases the size of the observed BOLD response. This difference can be increased either by producing a higher signal intensity during brain activation or by lowering the baseline signal intensity during the resting state.

The decreased resting cerebral blood flow due to the vasoconstrictor response might possibly account for the increased BOLD response after theophylline. The dose of theophylline used in this study reportedly produces a decrease of approximately 10% in the resting cerebral blood flow (6). If a constant level of oxygen utilization is assumed, a decreased resting cerebral blood flow should increase the resting deoxyhemoglobin concentration in the venous outflow, resulting in a decrease in the baseline signal intensity in the brain. If the increased rCBF with activation causes the signal intensity during activation to increase to the same or similar levels as the pretheophylline baseline level, the result is an increased difference between the lowered resting state and the normal or near-normal levels of activated signal intensity (Fig 1B). This increased difference would increase the observed BOLD response.

Recently, similar mechanism was proposed to account for the effects of anesthesia on the functional cerebral blood flow (fCBF) response to forepaw stimulation. Similar to the BOLD response, the fCBF response is determined by comparing cerebral blood flow during rest versus activation states. Hyder et al (26), have shown that the fCBF response during deep anesthesia is larger than the fCBF response during light anesthesia. This difference, they postulated, is due to changes in the resting cerebral blood flow; the resting blood flow during deep anesthesia is substantially lower than that during rest or light anesthesia.

Prior studies in which the BOLD response was perturbed have revealed a complex, sometimes confusing, picture. For example, hypercapnia is associated with vasodilation and increased cerebral blood flow. Some groups have shown that hypercapnia increases the BOLD response (27–30), whereas other groups suggest no significant change is observed (31). On the other hand, some have shown that hypocapnia increases the BOLD response (32), and others have shown that it decreases BOLD contrast (33). The reasons for these differences are not clear; they may be related to the experimental technique, the duration of hypercapnia and/or hypocapnia, or the specific brain location studied. The complexity of these findings suggests a multifactorial mechanism in which a given perturbation may affect several underlying physiologic processes.

Theophylline also has known neuroexcitatory properties that could potentially account for the increased BOLD response. Theophylline is an antagonist of the inhibitory neurotransmitter, adenosine, and it has been shown to increase the evoked potential levels in brain sections (34). With such a neuroexcitatory effect, theophylline could increase the number of neurons that are activated in response to a given forepaw sensory stimulus. An increase in the number of activated neurons could increase the volume of tissue activated. With a volume averaging effect, the BOLD response amplitude could appear larger than that of pretheophylline activation. This mechanism is supported in a recent report by Rees et al (35), who showed that the level of BOLD contrast is directly proportional to the average neuronal firing rate.

A paradoxical increase in cerebral blood flow during activation is unlikely to have caused our results. We monitored blood pressure, arterial P_{O_2} and P_{CO_2} throughout the experiments. These physiological measures remained stable and were not significantly different between the groups (Table). Additionally, theophylline has been shown to substantially attenuate cortical blood flow and pial vessel dilation during sciatic nerve stimulation in the rat (7); it also significantly decreases the cerebral blood flow response to whisker stimulation in rat whisker barrel cortex (17). However, we did not obtain dedicated MR flow measurements to directly quantify the effect of theophylline on the rCBF.

In this study, augmentation of the BOLD response after systemic theophylline administration was both robust and reproducible. We proposed several possible mechanisms to account for these observations. Additional experimental measurements, such as measurement of the changes in rCBF and resting deoxyhemoglobin level caused by theophylline, are required to distinguish between these possibilities.

Conclusion

With an experimental rat model, the BOLD response can be substantially increased by using a systemically administered drug. Potentially, experiments such as these can help in improving our understanding of the physiologic processes involved with cerebral activation. In addition, an increase in the BOLD re-

sponse can potentially increase the sensitivity of the fMRI technique. This improvement could allow the detection of more subtle activation patterns, and it may prove useful in applications that involve subtle alterations in neural activation, as in the study of cognitive tasks, neuropsychiatric disorders, and brain organization.

References

1. Malonek D, Dirnagel U, Lindauer U, Yamada K, Kanno I, Grinvald A. **Vascular imprints of neural activity: relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation.** *Proc Natl Acad Sci U S A* 1997;94:14826–14831
2. Ogawa S, Menon RS, Tank DW, et al. **Functional brain imaging by blood oxygenation level-dependent contrast magnetic resonance imaging.** *Biophys J* 1993;64:803–812
3. Ogawa S, Lee TM, Kay AR, Tank DW. **Brain magnetic resonance imaging with contrast dependent on blood oxygenation.** *Proc Natl Acad Sci U S A* 1990;87:9868–9872
4. Poulsen SA, Quinn RJ. **Adenosine receptors: new opportunities for future drugs.** *Bioorg Med Chem* 1998;6:619–641
5. Wahl M, Schilling L. **Regulation of cerebral blood flow: a brief review.** *Acta Neurochir Suppl (Wien)* 1993;59:3–10
6. Morii S, Ngai AC, Ko KR, Winn HR. **Role of adenosine in regulation of cerebral blood flow: effects of theophylline during normoxia and hypoxia.** *Am J Physiol* 1987;253:H165–H175
7. Ko KR, Ngai AC, Winn HR. **Role of adenosine in regulation of regional cerebral blood flow in sensory cortex.** *Am J Physiol* 1990; 259:H1703–H1708
8. Meno JR, Crum AV, Winn HR. **Effect of adenosine receptor blockade on pial arteriolar dilation during sciatic nerve stimulation.** *Am J Physiol* 2001 Nov;281:H2018–H2027
9. Morii S, Ngai AC, Winn HR. **Reactivity of rat pial arterioles and venules to adenosine and carbon dioxide: with detailed description of the closed cranial window technique in rats.** *J Cereb Blood Flow Metab* 1986;6:34–41
10. Hyder F, Behar KL, Martin MA, Blamire AM, Shulman RG. **Dynamic magnetic resonance imaging of the rat brain during forepaw stimulation.** *J Cereb Blood Flow Metab* 1994;14:649–655
11. Morton DW, Maravilla KR, Meno JR, Winn HR. **A clinically relevant rat model for testing BOLD fMRI techniques using single shot EPI at 1.5T.** *Radiology* 2001;218:598–601
12. Cohen JD, MacWhinney B, Flatt M, and Provost J. **PsyScope: a new graphic interactive environment for designing psychology experiments.** *Behav Res Methods Instrum Comput* 1993;25:257–271
13. Gyngell ML, Bock C, Schmitz B, Hoehn-Berlage M, Hossmann KA. **Variation of functional MRI signal in response to frequency of somatosensory stimulation in alpha-chloralose anesthetized rats.** *Magn Reson Med* 1996;36:13–15
14. Mandeville JB, Marota JJA, Kosofsky BE, et al. **Dynamic functional imaging of relative cerebral blood volume during rat forepaw stimulation.** *Magn Reson Med* 1998;39:615–624
15. Hsu EW, Hedlund LW, MacFall JR. **Functional MRI of the rat somatosensory cortex: effects of hyperventilation.** *Magn Reson Med* 1998;40:421–426
16. Li J, Iadecola C. **Nitric oxide and adenosine mediate vasodilatation during functional activation in cerebellar cortex.** *Neuropharmacology* 1994;33:1453–1461
17. Dirnagl U, Niwa K, Lindauer U, Villringer A. **Coupling of cerebral blood flow to neuronal activation: role of adenosine and nitric oxide.** *Am J Physiol* 1994;267:H296–H301
18. Kastrup A, Li TQ, Takahashi A, Glover GH, Moseley ME. **Functional magnetic resonance imaging of cerebral blood oxygenation changes during breath holding.** *Stroke* 1998;29:2641–2645
19. Rostrup E, Larsson HB, Toft PB, et al. **Functional MRI of CO2 induced increases in cerebral perfusion.** *NMR Biomed* 1994;7:29–34
20. Rostrup E, Law I, Blinkenberg M, et al. **Regional differences in the CBF and BOLD responses to hypercapnia: a combined PET and fMRI study.** *Neuroimage* 2000;11:87–97
21. Graham GD, Zhong J, Petroff OA, Constable RT, Prichard JW, Gore JC. **BOLD MRI monitoring of changes in cerebral perfusion induced by acetazolamide and hypercarbia in the rat.** *Magn Reson Med* 1994;3:557–560
22. Phillis JW, Delong RE. **An involvement of adenosine in cerebral blood flow regulation during hypercapnia.** *Gen Pharmacol* 1987;18: 133–139
23. Estevez AY, Phillis JW. **Hypercapnia-induced increases in cerebral blood flow: roles of adenosine, nitric oxide and cortical arousal.** *Brain Res* 1997;758:1–8
24. Hedera P, Lai S, Lewin JS, et al. **Assessment of cerebral blood flow reserve using functional magnetic resonance imaging.** *J Magn Reson Imaging* 1996;6:718–725
25. Bruhn H, Kleinschmidt A, Boecker H, Merboldt KD, Hanicke W, Frahm J. **The effect of acetazolamide on regional cerebral blood oxygenation at rest and under stimulation as assessed by MRI.** *J Cereb Blood Flow Metab* 1994;742–748
26. Hyder F, Shulman RG, Rothman DL, Behar KL. **Localized energetic changes with brain activation from anesthesia: absolute CBF changes at 7 tesla.** In: *Eighth Scientific Meeting and Exhibition: Book of Abstracts.* Berkeley, Calif: International Society for Magnetic Resonance in Medicine; 2000:8:444
27. Graham GD, Zhong J, Petroff OA, Constable RT, Prichard JW, Gore JC. **BOLD MRI monitoring of changes in cerebral perfusion induced by acetazolamide and hypercarbia in the rat.** *Magn Reson Med* 1994;31:557–560
28. Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. **Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model.** *Magn Reson Med* 1999;42:849–863
29. Kastrup A, Kruger G, Glover GH, Neumann-Haefelin T, Moseley ME. **Regional variability of cerebral blood oxygenation response to hypercapnia.** *Neuroimage* 1999;10:675–681
30. Rostrup E, Law I, Blinkenberg M, et al. **Regional differences in the CBF and BOLD responses to hypercapnia: a combined PET and fMRI study.** *Neuroimage* 2000;11:87–97
31. Li TQ, Kastrup A, Mosley ME, Glover GH. **Changes in baseline cerebral blood flow in humans do not influence regional cerebral blood flow response to photic stimulation.** *J Magn Reson Med* 2000;12:757–762
32. Hsu EW, Hedlund LW, MacFall JR. **Functional MRI of the rat somatosensory cortex: effects of hyperventilation.** *Magn Reson Med* 1998;40:421–426
33. Weckesser M, Posse S, Olthoff U, Kemna L, Dager S, Muller-Gartner HW. **Functional imaging of the visual cortex with bold-contrast MRI: hyperventilation decreases signal response.** *Magn Reson Med* 1999;41:213–216
34. Phillis JW, Edstrom JP, Kostopoulos GK, Kirkpatrick JR. **Effects of adenosine and adenine nucleotides on synaptic transmission in the cerebral cortex.** *Can J Physiol Pharmacol* 1979;57:1289–1312
35. Rees G, Friston K, Koch C. **A direct quantitative relationship between the functional properties of human and macaque V5.** *Nature Neurosci* 2000;3:716–723