Original Research

Pharmacological Modulation of the BOLD Response: A Study of Acetazolamide and Glyceryl Trinitrate in Humans

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Purpose: To examine the effect of acetazolamide, known to increase cerebral blood flow (CBF) and glyceryl trinitrate (GTN), known to increase cerebral blood volume (CBV) on the blood oxygenation level-dependent (BOLD) response in humans using 3 T magnetic resonance imaging (MRI), and to evaluate how pharmacological agents may modulate cerebral hemodynamic and thereby possibly the BOLD signal.

Materials and Methods: Six subjects were randomly allocated to receive acetazolamide, GTN, or placebo in a double-blind three-way crossover controlled study. Before, during, and after drug administration we recorded the BOLD response during visual stimulation with reversing checkerboard.

Results: We found that acetazolamide caused significant depression of the BOLD response ($P = 0.0066$). The maximum decrease occurred at 5 minutes after infusion and was 51.9% (95% confidence interval [CI], 22.03–81.76). GTN did not influence the BOLD response ($P = 0.55$).

Conclusion: The BOLD response is decreased during increased CBF by acetazolamide, suggesting an inverse relationship between global CBF and the BOLD response. GTN does not change the BOLD response. This indicates that GTN exerts an effect on the large vessels only and that CBV changes in the microvascular system are necessary to alter the BOLD response.

Key Words: acetazolamide; BOLD; cerebral blood flow (CBF); cerebral blood volume (CBV); fMRI; glyceryl trinitrate (GTN)
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BLOOD OXYGEN LEVEL-DEPENDENT (BOLD) functional magnetic resonance imaging (fMRI) is a widely used technique to indirectly study brain activity (1,2). A range of physiological parameters may influence the BOLD response, the most important being cerebral blood flow (CBF), cerebral blood volume (CBV), cerebral oxidative metabolic rate ($CMRO_2$), and the regional anatomy of the microvasculature (3). Pharmacological agents may modulate cerebral hemodynamic and thereby the BOLD response. Before changes in the BOLD response can be interpreted as changes in brain activity per se, the influence of CBF and CBV changes on the BOLD response should be better clarified. Additionally, if the BOLD response is used in translational studies employing a pharmacological perturbation to induce physiological changes, the effect of the pharmacological perturbation alone must be well characterized. It is well established that acetazolamide is a potent carbonic anhydrase (CA) inhibitor (4). Acetazolamide is believed to cause an increase in CBF without an effect on CBV (4,5), although there are studies that disagree (6). There is some controversy regarding the effect of acetazolamide on the BOLD response. The most common opinion is that acetazolamide causes a depression of the BOLD response (7–9), The nitric oxide (NO) donor glyceryl trinitrate (GTN) is known to increase CBV (10) and according to previous studies does not affect CBF (10–12). The effect of GTN on the BOLD response has not previously been studied.

We conducted a double-blind, placebo-controlled, three-way crossover study to explore the effect of acetazolamide and GTN on the BOLD response. We hypothesized that (1) intravenous administration of

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acetazolamide would decrease the BOLD response and that (2) intravenous (iv) administration of GTN would increase the BOLD response.

MATERIALS AND METHODS

Subjects

We recruited six healthy volunteers (3 women, 3 men). The mean age was 24 years (range, 21–31 years) and mean weight was 75.5 kg (range, 67–85 kg). Exclusion criteria were: a history of serious somatic disease; migraine or any other type of headache (except episodic tension-type headache less than once a month); previously abnormal potassium and calcium blood levels; glaucoma; allergic reaction to sulfonamides; daily intake of any medication except contraceptives; heavy caffeine users. All female participants used safe contraceptive methods. On the day of enrolment, physical and neurological examination, electrocardiography, urine-testing, and blood sampling were done. All participants gave informed consent to participate. The Ethical Committee of Copenhagen (KA-20060084) approved the study, which was conducted in accordance with the Helsinki II Declaration of 1964, as revised in Edinburgh in 2000.

Design and Randomization

The study was a placebo-controlled, double-blinded, three-way crossover study. The subjects were scanned on three different occasions with at least 1 week in between to avoid carry-over and period effect. On each visit the subjects received GTN (bolus with isotonic saline followed by 0.5 mg/kg/min GTN infusion over 20 minutes), acetazolamide (bolus with 1 g acetazolamide followed by isotonic saline infusion over 20 minutes), or placebo (bolus with isotonic saline followed by isotonic saline infusion over 20 minutes) in randomized fashion. Acetazolamide bolus produced an effective mean dose of 13.2 mg/kg (range, 11.7–14.9 mg/kg). Each subject received a dose sufficient to completely inhibit carbonic anhydrase activity in most tissue (4).

Experimental Design

All subjects reported to the laboratory headache-free. Coffee, tea, cocoa, or other methylxanthine-containing foods or beverages, and tobacco were not allowed for at least 12 hours before the start of the study. Subjects were placed in the scanner and a venous catheter (Venflon) was inserted into the left antecubital vein for bolus and infusion. Before drug administration we collected blood samples to determine the baseline hematocrit, potassium, and sodium levels. Electrocardiogram (ECG), end-tidal CO_2, blood oxygen saturation (pulse oxymeter), and heart rate were monitored continuously during the study. All measurements were preformed using Veris monitor (Medrad, Pittsburgh, PA), except for the end-tidal CO_2, which was monitored using a capnograph (Datex, Helsinki, Finland).

MRI was performed on a 3.0 T Philips Achieva Scanner (Philips Medical Systems, Best, The Netherlands) using an eight-element phased-array receive head coil. We first obtained a reference anatomical whole-brain image and then repeatedly measured the BOLD response after visual stimulation with a reversing checkerboard. We defined time of drug administration as T_0. The anatomical image was recorded at −15 minutes, BOLD response at −10, 5, 25, 45, 55 minutes. Blood pressure, heart rate, ECG, end-tidal CO_2, blood oxygen saturation, and adverse events (AEs) such as headache, nausea, and other sensations relating to drug side-effects were recorded at −10, 0, 30, 45, and 75 minutes.

Data Acquisition and Imaging Protocols

Anatomical Images

Anatomical images were acquired using a T1-weighted 3D turbo field echo sequence (170 sagittal slices 1 mm thick; in-plane resolution 1 × 1 mm: repetition time 9.9 sec; echo time 4.6 msec; flip angle 8°).

BOLD Response

BOLD functional imaging utilized a gradient echo EPI sequence (32 slices 4.0 mm thick; slice gap 0.1 mm; field of view 230 × 230 mm; in-plane acquired resolution 2.9 × 2.9 mm; repetition time 3.0 sec; echo time 35 msec; flip angle 90°; SENSE factor 2). Slices were oriented parallel with the inferior border of corpus callosum covering the whole brain. The first four volumes of each run were discarded to avoid saturation effects. We obtained 100 volumes during each 5-minute scan session. To record the BOLD response we applied visual stimulation with a checkerboard. This is a well-established modality that produces a rather large BOLD response in the visual cortex (13). Visual stimulation was presented with the Eloquence system (Invivo, Orlando, FL), using a pair of NNL goggles (NordicNeuroLab, Bergen, Norway). A fiber optic cable connected the system to a control computer outside the scanner room. The paradigm consisted of rest blocks, where a uniform gray image was shown, alternating with active blocks displaying a black and white checkerboard reversing at 8 Hz. The block length was 1 minute and two activation periods were included during a scan session which had a duration of 5 minutes. Subjects were asked to fixate on a central fixation cross during the entire scan. The onset of visual stimuli was triggered by the scan acquisition.

Data Analysis and Statistics

All values are presented as mean ± SD and hemodynamic peak responses as mean percentage from baseline (95% confidence interval [CI]). The incidence of AEs is presented. Baseline was defined as T_{-10}.

The primary endpoint was difference in relative BOLD response between active drug and placebo. The secondary endpoint was difference in incidence of AEs between experimental days. We tested for period and carry-over effects for all baseline hemodynamic variables using independent Student's *t*-test. McNemar's test was used to test difference in incidence of AEs

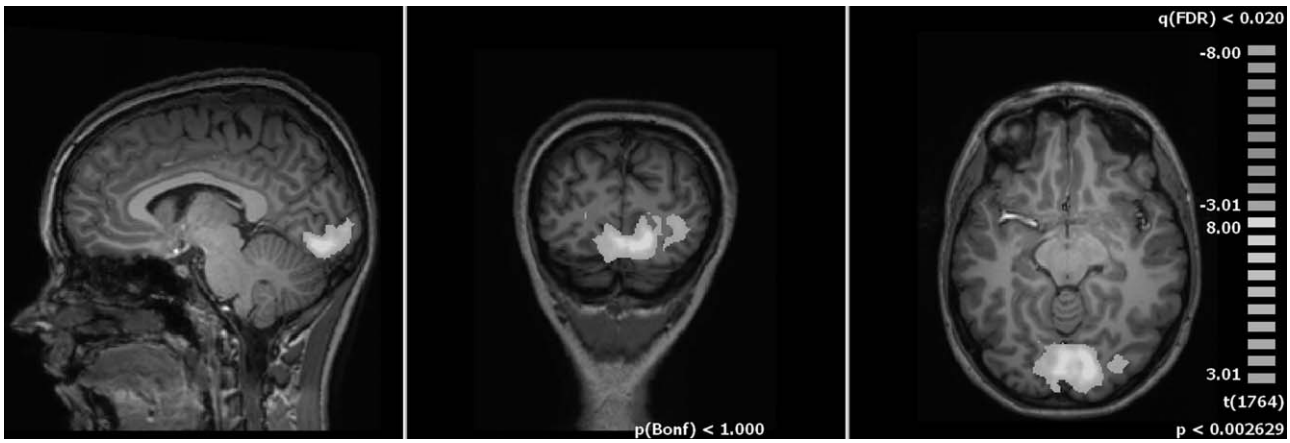

Figure 1. ROI for visual stimulation as defined by conjunction of all individual's activation areas in the first scan.

between experimental days. Five percent ($P < 0.05$) was accepted as the level of significance.

BOLD Data

The analysis was performed using BrainVoyager 1.9 (Brain Innovation, Maastricht, The Netherlands). Image preprocessing started with a 3D rigid-body motion correction and correction for slice-time. After 3D spatial smoothing with a 6-mm full-width at half-maximum (FWHM) Gaussian kernel, the functional images were coregistered to the 3D anatomies, transformed into Talairach space, and resampled to $3 \times 3 \times 3$ mm^3 voxels.

The statistical analysis used a general linear model. The visual block stimulation paradigm convolved with a 2 gamma variate hemodynamic response function served as a model time course. In a second-level analysis a two-way analysis of variance (ANOVA) of the BOLD effect size (beta-values) was performed with regard to scan-time and drug. GTN versus placebo and acetazolamide versus placebo was analyzed separately. The F-test statistic for the interaction of scan-time and drug was calculated both voxel-wise and for a region-of-interest (ROI). An ROI for the visual stimulation was defined by a conjunction of activation areas in all individuals from the first scan (before drug administration), which were in turn determined by requiring a false discovery rate (FDR) $P < 0.02$ to correct for multiple comparisons (Fig. 1).

RESULTS

All six subjects completed the study on all 3 study days. There were no missing data. We found no significant carry-over or period effect for BOLD response, blood pressure, or heart rate ($P > 0.05$). Baseline blood samples showed normal hematocrit, potassium, and sodium levels.

Acetazolamide and BOLD

Voxel-vise ANOVA did not show significantly activated voxels Two-factors ANOVA on the repeated measurement beta-values obtained from the ROI revealed a significant interaction between acetazolamide and placebo ($P = 0.011$). Further analysis showed significant changes over time after acetazolamide ($P = 0.0066$) but not after placebo ($P = 0.74$). Post-hoc pairwise t-tests showed a significant decrease in BOLD response at 5 minutes ($P = 0.001$), 25 minutes ($P = 0.05$), and 45 minutes ($P = 0.003$) after acetazolamide administration compared to baseline. The BOLD response did not return to baseline during the observation period but the last recording at 55 minutes did not show statistical difference compared to baseline ($P = 0.092$). The maximum decrease of 51.9% (95% CI, 22.0–81.8) occurred at 5 minutes (Fig. 2).

After acetazolamide administration we observed a time shift with regard to the effect on the BOLD response. This means that the BOLD response reached its peak and returned to rest later when compared to

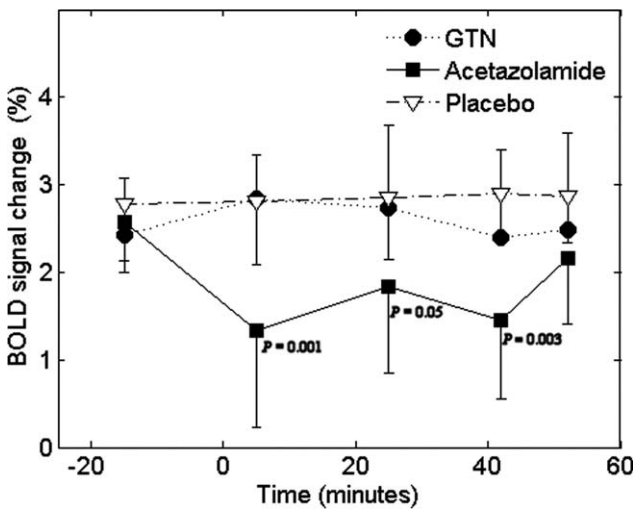

Figure 2. BOLD percent changes as function of measurement time relative to the time of start of injection. Acetazolamide significantly depressed BOLD responses at 5 minutes ($P = 0.001$), 25 minutes ($P = 0.05$), and 45 minutes ($P = 0.003$) compared to baseline. We found no statistically significant change in BOLD responses after GTN or placebo. Error bars are standard deviations.

Table 1
Incidence of Adverse Events

Adverse events	Acetazolamide	GTN	Placebo
Headache	5 ($P = 0.043$)	4 ($P = 0.066$)	0
Nausea	1 *	2*	0
Heat sensation	1 *	1*	0
Sensitivity to sound	1 *	2*	1

P-values show the difference between acetazolamide and placebo, and between GTN and placebo (McNemar's test).
*Nonsignificant difference, $P > 0.05$.

after placebo and GTN. This phenomenon was recorded at 5, 25, 45, and 55 minutes (Fig. 2).

GTN and BOLD Response

The voxel-vise ANOVA did not show significant voxel activation. Two-factor ANOVA on the repeated beta-value measurements obtained from the ROI revealed no interaction between GTN and placebo ($P = 0.45$) (Fig. 2).

Adverse Events

The incidence AEs is reported in Table 1.

Vascular Data

The vascular data including end-tidal CO_2 are reported in Table 2. There was no difference in baseline values between experimental days ($P > 0.05$).

DISCUSSION

The major finding was that, contrary to our prediction, GTN administration did not have any effect on the BOLD response. However, the BOLD response did decrease after administration of acetazolamide and did not return to baseline during the observation period (0–55 minutes). The peak decrease occurred 5 minutes after acetazolamide administration.

Acetazolamide is a potent CA inhibitor. Acetazolamide exerts no detectable toxic effect at physiologically useful doses (<50 mg/kg) and is believed to alter CBF through a transient arterial acidosis by inhibiting CA and thereby cause an increase in CBF by 20%–40% according to single photon emission tomography (SPECT) (4), positron emission tomography (PET) (14), and arterial spin labeling (ASL) fMRI (7) studies. The CBF increase is probably caused by a dilatation of arterioles due to the induced pH changes (15). A SPECT study reported that acetazolamide induced a rapid and marked increase in CBF without changes in $CMRO_2$ and suggested no effect of acetazolamide on CBV (4). However, two studies reported CBV changes in healthy volunteers after acetazolamide administration (9,14). Yamauchi et al (9) performed PET and dynamic contrast enhanced susceptibility (DSC) MRI perfusion measurements in healthy subjects and found that MRI overestimated both CBF and CBV. However, it is now generally agreed that reliable values of CBF and CBV are difficult to obtain with DSC MRI (16). Okazawa et al (14) found CBF and CBV increases following acetazolamide administration, but concluded that the CBV increase was probably due to dilatation of the large arteries. This is still unclear and a subject of debate.

In the present study we used a bolus dose of acetazolamide sufficient to block CA (4). We found that acetazolamide caused a depression of the BOLD response, which is in agreement with previous studies (7,8,17). Two studies reported a decrease in BOLD responses in healthy volunteers after a hand motor task ($n = 5$) (7), and during a breath-holding task ($n = 17$) (17). In the third study BOLD images were obtained in five anesthetized rats after acetazolamide administration (8). In addition, two studies reported an increased BOLD signal amplitude but a smaller BOLD response after acetazolamide during a hand motor task in four patients with unilateral occlusion of the internal carotid artery (18) and during a visual task in six healthy volunteers (19). There are, however, some important methodological reservations about the results from these studies. Thus, the authors reported large intersubject variability without presenting *P*-values in the Results section and both studies were not randomized or placebo-controlled. In the present study we observed a maximum decrease in BOLD response 5 minutes after acetazolamide, even though after acetazolamide bolus, the drug attains peak plasma levels within 15 to 18 minutes (4). The present data suggest that a much smaller dose is sufficient to completely block CA. The halftime of intravenous acetazolamide is ≈1.7 hours (20). This could explain why the BOLD response continued to be depressed during the observation period. It should be noted, however that we found no statistical difference when comparing the recorded BOLD response to baseline (before drug administration) or to placebo at the last 55 minutes recording.

In conclusion, we found that acetazolamide depressed the BOLD response probably by increasing CBF. This interpretation is supported by studies using different pharmacological substances to examine the relationship between the BOLD response and CBF. Two studies reported that caffeine caused a lowering of CBF (21,22) and Mulderink et al (21) reported a subsequent increase in the BOLD response. Alcohol increases global CBF (23) while causing a depression of the BOLD response (24). Furthermore, Kruuse et al (25) reported that sildenafil neither changed CBF nor BOLD response in healthy subjects (26). Taken together, the

Table 2
Mean End-Tidal P_{CO2}, Blood Pressure, Heat Rate, and O_2 Saturation

Measured parameters	Mean ± SD
End-tidal P_{CO2} (kPa)	4,77 ± 0.3
BP systolic (mm Hg)	114 ± 11
BP diastolic (mm Hg)	65 ± 8.4
Heart rate (bpm)	70 ± 15
O_2 saturation (percent)	98 ± 1

previous and present data further add to the growing evidence of dependency between CBF and the elicited BOLD response, and suggest an inverse relationship between CBF and the BOLD response (27).

Surprisingly, we found no changes in BOLD response after GTN. The effect of nitric oxide (NO) donor GTN on the BOLD response has not previously been examined. The vascular actions of NO include both a direct and an indirect vasodilatation (28). NO readily passes through the blood–brain barrier and into the smooth muscle cells resulting in activation of cGMP, smooth muscle relaxation in the arteries, and vasodilatation (29). It seems that that GTN does not change CBF (10–12). One study reported that infusion of GTN (0.5 mg/kg/min in 20 minutes) solely increased CBV by 13% without effecting CBF (10). In this study CBV was measured by 99mTC-labeled erythrocytes and CBF was measured by following the 133-xenon brain washout curve after an abrupt halt of xenon inhalation using SPECT. Notably, a rather low spatial resolution was employed (16 mm in the horizontal plane). Mean velocity measured by transcranial Doppler in the medial cerebral artery (MCA) showed a 20% decrease, indicating a vasodilatation. These data could have important consequences for BOLD imaging, because a neural activity-dependent CBF increase on top of an already expanded CBV could hypothetically result in a higher BOLD response. Based on estimations of microvascular morphometry it is believed that the majority of blood at baseline is partitioned in the venous compartment (46%), with the remaining being partitioned in the capillaries (33%) and arterial compartments (21%) (30). According to animal studies the respective compartments are affected unequally by CBV changes induced by hypo- or hypercapnia and by somatosensory stimulation—where the majority of changes affect the venous compartment (36%–62% depending on the sources) and only to a lesser extent the arterial and capillary compartments (31,32). Therefore, given that ΔR2 is proportional to venous cerebral blood volume (33) and given that the GTN induced CBV changes primarily increases the venous CBV, we expected to find an increased BOLD response during neural activation. However, no changes in BOLD response were observed. We can interpret our results in the framework of a widely used model for the relationship between the BOLD response on the one hand, and CBF, CBV, and the cerebral metabolic rate of oxygen ($CMRO_2$) on the other (23,24). Briefly, the contribution of the concentration of venous deoxyhemoglobin [dHb] to the transverse relaxation rate R_2* is (34):

$$R_2^*|_{dHb} = A \cdot CBV \cdot [dHb]^{\beta}. \quad [1]$$

Here A and β are constants and $1 \leq \beta \leq 2$. If S_0 is the MR signal in the resting situation and ΔS is the change upon stimulation, then the BOLD response is:

$$\frac{\Delta S}{S_0} \cong -TE \cdot \Delta R_2^*|_{dHb} = TE \cdot A(CBV_0[dHb]_0^{\beta} - CBV[dHb]^{\beta}). \quad [2]$$

The approximation is valid for small R_2* changes. Finally, employing Fick's principle (35,36), we arrive at:

$$\frac{\Delta S}{S_0} = -TE \cdot A \cdot 4^{-\beta}[CBV_0(CMRO_{2,0}/CBF_0)^{\beta} - CBV(CMRO_{2,0}/CBF_0)^{\beta}] \quad [3]$$

It can be shown from Eq. [3] that if resting flow (CBF_0) increases while the flow change upon stimulation (CBF-CBF_0) is constant as well as all blood volume and metabolism parameters, then $\Delta S/S_0$ decreases, in accordance with our findings regarding acetazolamide. On the other hand, if resting blood volume (CBV_0) increases, and (CBV-CBV_0) as well as all flow and metabolism parameters are constant, then $\Delta S/S_0$ is expected to increase, which does not agree with our findings regarding GTN. These conclusions are also true if the fractional blood flow (CBF/CBF_0) or blood volume (CBV/CBV_0) changes are assumed constant across pharmacological challenges, instead of absolute flow and volume changes.

However, the assumption that CBF_0 and CBV_0 are not coupled is not supported by previous studies, which have utilized various forms of hyper- or hypocapnia to study the interrelation between CBV and CBF in animals (32,37) and humans (38,39). Most notable is this relationship between CBF and CBV described by Grubb et al. (40), who found that:

$$CBV = 0.8 \cdot CBF^{\alpha}. \quad [4]$$

where α was found to be 0.38. According to these principles an isolated effect on CBV, without a concomitant effect on CBF, is not possible. Therefore, there is a mismatch between previous studies often based on nuclear medicine methods (10–12), and our current understanding of the primary determinants of the BOLD response.

If Eq. [4] is further inserted into Eq. [3], then the BOLD response is:

$$\frac{\Delta S}{S_0} = 0.8 \cdot TE \cdot A \cdot 4^{-\beta}(CBF_0^{\alpha-\beta}CMRO_{2,0}^{\beta} - CBF^{\alpha-\beta}CMRO_2^{\beta}) = 0.8^{\beta/\alpha} \cdot TE \cdot A \cdot 4^{-\beta}(CBF_0^{1-\beta/\alpha}CMRO_{2,0}^{\beta} - CBF^{1-\beta/\alpha}CMRO_2^{\beta}) \quad [5]$$

As before, if resting flow (CBF_0) increases, and the flow change upon stimulation (CBF-CBF_0) is constant, then $\Delta S/S_0$ decreases, in accordance with our findings regarding acetazolamide. However, if CBV_0 increases and (CBV-CBV_0) is constant, the BOLD response $\Delta S/S_0$ will decrease. This predicted behavior arises because of the coupling of blood flow and volume through Eq. [4], and is opposite to that of an isolated CBV_0 change, discussed above. Still, the model does not agree with our findings regarding GTN. If, alternatively, fractional blood flow or blood volume changes are assumed constant, the same conclusions are reached.

To quantify the above considerations, we assumed values of the parameters in Eq. [5] from the literature.

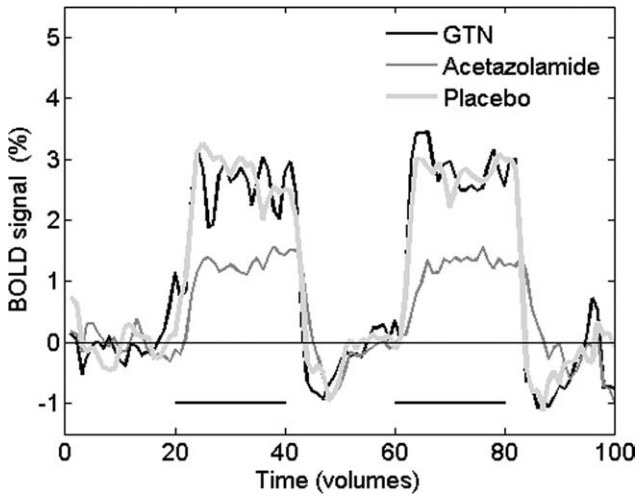

Figure 3. The largest change in BOLD response after acetazolamide at 5 minutes in a visual ROI. Acetazolamide depressed BOLD responses and returned to baseline later than after GTN and placebo.

We used $\alpha = 0.38$ (39), $\beta = 1.5$ (33), and $CMRO_2/CMRO_{2,0} = 1.25$ (35). We further assumed $CBF_0 = 60$ mL/100g/min and $CBF/CBF_0 = 1.5$ (35) in the absence of an active drug and used either a constant additive or proportional blood flow change. The prefactor in Eq. [5] was found from the BOLD response before drug administration, and from the BOLD response at later timepoints we deduced the change in CBF_0 (and thereby also the change in CBV_0 using Eq. [5]) due to administration of acetazolamide or GTN. This was done for each subject separately. For acetazolamide, we focused on the timepoint at 5 minutes (see Fig. 3), where the largest change in BOLD response was observed. We found an average CBF_0 increase of 51.1%, which was statistically significant ($P = 0.04$) assuming an additive flow change, and a nonsignificant average CBF_0 increase of 134.0%, assuming a proportional flow change (4,5,7). The additive model gives the best agreement with the literature, but a further comparison of the additive and proportional models is not within the scope of the present work. For GTN, the change in blood volume was not significant at any timepoint, regardless of whether a constant additive or proportional flow change was assumed. The average change of CBV_0 across subjects ranged from −5.5% to +4.5% depending on time and model assumptions. To investigate if the lack of detectable effect of GTN on CBV_0 was due to a specific choice of model parameters, we repeated the calculation for parameters $\alpha = 0.3$ to 0.5, $\beta = 1$ to 2, $CMRO_2/CMRO_{2,0} = 1.1$ to 1.4, and $CBF/CBF_0 = 1.3$ to 1.7; with 41 values of every parameter. For some parameter combinations the measured BOLD responses could not be modeled; such parameter combinations were disregarded. For none of the remaining parameter combinations, models, or timepoints was the CBV_0 change significant.

The BOLD response is dependent on deoxygenation changes in the venoules and venous part of the capillaries (32,36). One possible explanation for the lack of effect of GTN on the BOLD response could be that GTN affects the macrovascular part of the vascular system and does not have an effect on the microvascular system that is involved in the BOLD mechanism. Thus, we speculate that the lowering of the velocity in MCA after GTN suggests that large arteries dilate, and likely lengthen a bolus arrival time in a contrast-based perfusion study. However, this does not change the perfusion itself, because GTN is unlikely to dilate the resistance vessels. Furthermore, GTN may dilate large venous vessels, without affecting the venoules where passive inflation is caused by elevation of the venous blood pressure occurring secondary to a lowering of the arteriolar resistance. Concerning acetazolamide, it undoubtedly increases CBF and thereby inflates small venoules without effect on the large venous compartment.

In conclusion, the present study demonstrates that the BOLD response is decreased during increased global CBF. Furthermore, it is likely that GTN exerts an effect on the large vessels only without affecting the microvascular compartment and therefore does not change the BOLD response. The BOLD fMRI allows sequential measurements in humans in response to a variety of pharmacological stimuli in health and disease. The present data underline the importance of further exploration of methodological aspects of interdependency and testing the influence of pharmacological substance on the BOLD response per se to avoid false-negative or false-positive results.

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REFERENCES

1. Kwong K, Belliveau J, Chesler D, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci U S A 1992;89:5675–5679.
2. Ogawa S, Lee T, Nayak A, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. Magn Reson Med 1990;14:68–78.
3. Buxton R, Wong E, Frank L. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. Magn Reson Med 1998;39:855–864.
4. Vorstrup S, Henriksen L, Paulson O. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. J Clin Invest 1984;74:1634–1639.
5. Bonte F, Devous M, Reisch J. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single-photon emission computed tomography. Invest Radiol 1988;23:564–568.
6. Grandin CB, Bol A, Smith AM, Michel C, Cosnard G. Absolute CBF and CBV measurements by MRI bolus tracking before and after acetazolamide challenge: repeatabilily and comparison with PET in humans. Neuroimage 2005;26:525–535.
7. Brown G, Eyler Zorrilla L, Georgy B, Kindermann S, Wong E, Buxton R. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J Cereb Blood Flow Metab 2003;23:829–837.
8. Mukherjee B, Preece M, Houston G, et al. Mapping of the cerebral response to acetazolamide using graded asymmetric spin echo EPI. Magn Reson Imaging 2005;23:907–920.

9. Yamauchi H, Okazawa H, Kishibe Y, Sugimoto K, Takahashi M. The effect of acetazolamide on the changes of cerebral blood flow and oxygen metabolism during visual stimulation. Neuroimage 2003;20:543–549.
10. Iversen H, Holm S, Friberg L, Tfelt-Hansen P. Intracranial hemodynamics during intravenous infusion of glyceryl trinitrate. J Headache Pain 2008;9:177–180.
11. Iversen H, Olesen J, Tfelt-Hansen P. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. Pain 1989;38:17–24.
12. White RP, Deane C, Hindley C, et al. The effect of the nitric oxide donor glyceryl trinitrate on global and regional cerebral blood flow in man. J Neurol Sci 2000;178:23–28.
13. Genovese C, Lazar N, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 2002;15:870–878.
14. Okazawa H, Yamauchi H, Sugimoto K, Toyoda H, Kishibe Y, Takahashi M. Effects of acetazolamide on cerebral blood flow, blood volume, and oxygen metabolism: a positron emission tomography study with healthy volunteers. J Cereb Blood Flow Metab 2001;21:1472–1479.
15. Travis DM, Wiley C, Nechay BR, Maren Th. Selective renal carbonic anhydrase inhibition without respiratory effect: pharmacology of 2-benzensulfonamide (CL 11,366). J Pharmacol Exp Ther 1964;143:383–394.
16. Kiselev VG. Transverse relaxation effect of MRI contrast agents: a crucial issue for quantitative measurements of cerebral perfusion. J Magn Reson Imaging 2005;22:693–696.
17. Shiino A, Morita Y, Tsuji A, et al. Estimation of cerebral perfusion reserve by blood oxygenation level-dependent imaging: comparison with single-photon emission computed tomography. J Cereb Blood Flow Metab 2003;23:121–135.
18. Kleinschmidt A, Steinmetz H, Sitzer M, Merboldt K, Frahm J. Magnetic resonance imaging of regional cerebral blood oxygenation changes under acetazolamide in carotid occlusive disease. Stroke 1995;26:106–110.
19. Bruhn H, Kleinschmidt A, Boecker H, Merboldt K, 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;14:742–748.
20. Maren Th, Robinson B. The pharmacology of acetazolamide as related to cerebrospinal fluid and the treatment of hydrocephalus. Bull Johns Hopkins Hosp 1960;106:1–24.
21. Mulderink T, Gitelman D, Mesulam M, Parrish T. On the use of caffeine as a contrast booster for BOLD fMRI studies. Neuroimage 2002;15:37–44.
22. Perthen J, Lansing A, Liau J, Liu T, Buxton R. Caffeine-induced uncoupling of cerebral blood flow and oxygen metabolism: a calibrated BOLD fMRI study. Neuroimage 2008;40:237–247.
23. Newlin D, Golden C, Quaife M, Graber B. Effect of alcohol ingestion on regional cerebral blood flow. Int J Neurosci 1982;17:145–150.
24. Levin J, Ross M, Mendelson J, et al. Reduction in BOLD fMRI response to primary visual stimulation following alcohol ingestion. Psychiatry Res 1998;82:135–146.
25. Kruuse C, Thomsen L, Jacobsen T, Olesen J. The phosphodiesterase 5 inhibitor sildenafil has no effect on cerebral blood flow or blood velocity, but nevertheless induces headache in healthy subjects. J Cereb Blood Flow Metab 2002;22:1124–1131.
26. Kruuse C, Hansen A, Larsson H, Lauritzen M, Rostrup E. Cerebral haemodynamic response or excitability is not affected by sildenafil. J Cereb Blood Flow Metab [Internet] 2009; Available at: PM:19209179.
27. Liau J, Liu TT. Inter-subject variability in hypercapnic normalization of the BOLD fMRI response. Neuroimage 2009;45:420–430.
28. Modin A, Björne H, Herulf M, Alving K, Weitzberg E, Lundberg JO. Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. Acta Physiol Scand 2001;171:9–16.
29. Archer SL, Huang JM, Hampl V, Nelson DP, Shultz PJ, Weir EK. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. Proc Natl Acad Sci U S A 1994;91:7583–7587.
30. van Zijl PC, Eleff SM, Ulatowski JA, et al. Quantitative assessment of blood flow, blood volume and blood oxygenation effects in functional magnetic resonance imaging. Nat Med 1998;4:159–167.
31. Mandeville JB, Marota JJ, Ayata C, et al. Evidence of a cerebrovascular postarteriole windkessel with delayed compliance. J Cereb Blood Flow Metab 1999;19:679–689.
32. Lee SP, Duong TQ, Yang G, Iadecola C, Kim SG. Relative changes of cerebral arterial and venous blood volumes during increased cerebral blood flow: implications for BOLD fMRI. Magn Reson Med 2001;45:791–800.
33. Cohen ER, Rostrup E, Sidaros K, et al. Hypercapnic normalization of BOLD fMRI: comparison across field strengths and pulse sequences. Neuroimage 2004;23:613–624.
34. Boxerman JL, Bandettini PA, Kwong KK, et al. The intravascular contribution to fMRI signal change: Monte Carlo modeling and diffusion-weighted studies in vivo. Magn Reson Med 1995;34:4–10.
35. Davis T, Kwong K, Weisskoff R, Rosen B. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. Proc Natl Acad Sci U S A 1998;95:1834–1839.
36. Hoge R, Atkinson J, Gill B, Crelier G, Marrett S, Pike G. Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. Magn Reson Med 1999;42:849–863.
37. Wu G, Luo F, Li Z, Zhao X, Li S. Transient relationships among BOLD, CBV, and CBF changes in rat brain as detected by functional MRI. Magn Reson Med 2002;48:987–993.
38. Chen JJ, Pike GB. BOLD-specific cerebral blood volume and blood flow changes during neuronal activation in humans. NMR Biomed 2009;22:1054–1062.
39. Rostrup E, Knudsen G, Law I, Holm S, Larsson H, Paulson O. The relationship between cerebral blood flow and volume in humans. Neuroimage 2005;24:1–11.
40. Grubb R, Raichle M, Eichling J, Ter-Pogossian M. The effects of changes in PaCO2 on cerebral blood volume, blood flow, and vascular mean transit time. Stroke 1974;5:630–639.