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The effect of acetazolamide on the changes of cerebral blood flow and oxygen metabolism during visual stimulation

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Abstract

Acetazolamide, a carbonic anhydrase inhibitor, has an anticonvulsant effect which may result from a decrease in the efficacy of synaptic transmission due to a decrease of pH. Our previous study showed that acetazolamide induced a significant increase in global and regional cerebral blood flow (CBF), but caused no significant change in the cerebral metabolic rate of oxygen ($CMRO_2$). To investigate the effect of acetazolamide on the responses of CBF and $CMRO_2$ during neural stimulation, we used positron emission tomography to measure CBF and $CMRO_2$ in six normal volunteers at the fixation-only baseline visual state and during visual stimulation before and after administration of 1 g of acetazolamide. Visual stimulation induced a significant increase in CBF (33%) in the visual cortex compared with baseline values, but caused no significant change in $CMRO_2$, while no significant change in global CBF or $CMRO_2$ was found. During visual stimulation after acetazolamide administration, both global and visual cortical CBF and $CMRO_2$ showed similar changes compared with the respective baseline values (37 and 65% increases in CBF and 8 and 16% decreases in $CMRO_2$, respectively). When corrected by the global values, the magnitudes of the CBF and $CMRO_2$ responses to visual stimulation after acetazolamide administration were less than those before (20% vs 38% in CBF and −9% vs 3% in $CMRO_2$). Considering our previous observation that the effect of acetazolamide was similar throughout cerebral cortical regions, we suggest that acetazolamide decreases the responses of both CBF and $CMRO_2$ during visual stimulation, which indicates that this drug may affect neuronal excitability.
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Keywords: Cerebral blood flow; Cerebral metabolic rate of oxygen; Acetazolamide; Positron emission tomography; Visual cortex

Introduction

During neural stimulation in the normal brain, increases in cerebral blood flow (CBF) are accompanied by lesser increases in the cerebral metabolic rate of oxygen ($CMRO_2$) (Fox and Raichle, 1986; Raichle, 1998). Consequently, local increases in brain oxygen content occur at the site of activation and provide the basis for the signal measured by functional magnetic resonance imaging (fMRI) (Ogawa et al., 1990; Raichle, 1998). This blood oxygenation level-dependent (BOLD) fMRI method has recently been extended to the investigation of brain conditions after the administration of centrally active drugs, and the findings suggest the usefulness of this method for elucidation of pharmacological effects on brain function (Tracey, 2001). Despite these advances, at present the relationship between neural activation-related changes in CBF and $CMRO_2$ under different physiological or pharmacological conditions is only partially understood. If the relationship between the CBF and $CMRO_2$ responses were to change, the interpretation of the neural activation-related BOLD signal change would become difficult, because the BOLD signal is the convolution of several components, including CBF, $CMRO_2$, and cerebral blood volume (CBV), and the change in $CMRO_2$/CBF provides a measure of the BOLD contrast (Ogawa et al., 1990; Vafaee and Gjedde 2000). However,

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most BOLD fMRI studies have neglected this possibility, because few studies have directly demonstrated the changes of the relationship between neural activation-related changes in CBF and $CMRO_2$ after the administration of drugs. If the BOLD response is to be used to measure drug-induced changes in neural activity, it will be necessary to demonstrate by direct measurements of CBF and $CMRO_2$ responses that the drug does not change the relationship between neural activation-related changes in CBF and $CMRO_2$.

Carbonic anhydrase inhibitors, including acetazolamide, have anticonvulsant effects that may result from a decrease in the efficacy of synaptic transmission due to a decrease in the extracellular and intracellular pH (Leniger et al., 2002). The magnitude of neuronal excitation after acetazolamide administration may be less than before, which may cause a parallel decrease in the responses of CBF and $CMRO_2$ during neural stimulation. Our previous study investigated the effect of acetazolamide on CBF and $CMRO_2$ in normal volunteers (Okazawa et al., 2001). Based on the former methodological work, the purpose of this study was to investigate the effect of acetazolamide on the responses of CBF and $CMRO_2$ during neural stimulation by using positron emission tomography (PET).

Subjects and methods

Subjects

We studied six normal volunteers, 26 ± 9 (mean ± SD) years old. Four of them were men. The subjects showed normal neurological findings and no specific neurologic diseases. None exhibited any abnormal MRI findings, except for a few punctate high-intensity areas in the subcortical white matter on T2-weighted images without corresponding abnormality on T1-weighted images. Written informed consent was obtained from each subject under the guidance of the Ethics Committee of the Shiga Medical Center.

PET measurements

All subjects underwent PET scans with a whole-body Advance (General Electric Medical System, Milwaukee, WI, USA) PET scanner, which permits simultaneous acquisition of 35 image slices with interslice spacing of 4.25 mm (DeGardo et al., 1994). Performance tests showed the intrinsic resolution of the scanner to be 4.6 to 5.7 mm in the transaxial direction and 4.0 to 5.3 mm in the axial direction. As part of the scanning procedure but before the tracer administration, $^{68}Ge/^{68}Ga$ transmission scanning was performed for 10 min for attenuation correction. For reconstruction of PET data, images were blurred to 6.0 mm full-width half-maximum in the transaxial direction using a Hanning filter. Functional images were reconstructed as 128 × 128 pixels, with each pixel representing an area of 2.0 × 2.0 mm.

The subjects were positioned in the scanner with their heads immobilized with a head-holder and positioned with light beams to obtain transaxial slices parallel to the orbitomeatal line. A small cannula was placed in the left brachial artery for blood sampling. After intravenous bolus injection of 555 MBq of $H_2{}^{15}O$ into the right antecubital vein, a 3-min dynamic PET scan was started at the time of tracer administration with frame durations of 5 s × 12, 10 s × 6, and 20 s × 3. In the oxygen bolus inhalation method, the same procedure of dynamic PET acquisition was started at the time of bolus inhalation of $^{15}O_2$, at an amount of up to 1800 MBq. Arterial blood was continuously drawn from a catheter in the radial artery with a pump, and the concentration of radioactivity was monitored with an in-line flow-through radioactivity detector, Pico-Count (Bioscan, Inc., Washington, DC, USA) (Votaw and Shulman, 1998) and the concentration of radioactivity was then used as an input function for data analysis. Arterial hematocrit, hemoglobin concentration, PaO_2, and $PaCO_2$ were also measured. CBF was calculated using the autoradiographic method with a partition coefficient of 0.9 (ml/g) (Herscovitch et al. 1983; Raichle et al., 1983). $CMRO_2$ was calculated from the dynamic PET data and arterial blood curves by using the three-weighted integral (3-WI) method based on a two-compartment model. The calculation procedure for the 3-WI method has been described in detail elsewhere (Ohta et al., 1992, 1996). The time delay of arterial input was corrected automatically in the program, and a time constant of $\tau = 4$ s was used for internal dispersion correction (Ohta et al., 1996).

Each subject underwent PET scans under three conditions: no stimulation (baseline), visual activation, and visual activation after acetazolamide administration. Under the baseline condition, the subjects were asked to fixate on a cross-hair in the center of the screen 30 s before the scan and throughout the subsequent 3-min scan. Under the visual activation condition after baseline scans, the subjects were shown a yellow–blue annular checkerboard whose contrast was reversed at a frequency of 4 Hz, which may cause a maximum change of $CMRO_2$ during visual stimulation (Vafaee et al., 1999). Visual stimulation began 4 min before the start of the dynamic PET scan and continued for a total of 7 min. After the scans during visual activation, 1 g of acetazolamide was administered intravenously (Okazawa et al., 2001). Ten minutes after the administration, a third $H_2{}^{15}O$ study was done in the same way as in the visual activation study. Twenty minutes after the administration, a third $^{15}O_2$ study was done. Our previous study showed that the effect of acetazolamide is likely to be constant over this period (Okazawa et al., 2001), but may decrease thereafter.

No subject showed a significant change in $PaCO_2$ during PET scanning, and the changes in the physiologic data during acetazolamide administration were small in all subjects (Table 1).

Table 1
Physiological data obtained under the baseline and activation conditions

Variable	Baseline	Visual activation	
		Before administration of acetazolamide	After administration of acetazolamide
Hematocrit (%)	40.6 ± 4.7	40.5 ± 4.9	41.2 ± 4.8
Hemoglobin (g/dl)	13.2 ± 1.5	13.1 ± 1.6	13.4 ± 1.6
$PaCO_2$ (mm Hg)	41.0 ± 3.0	42.1 ± 2.2	41.3 ± 1.0
PaO_2 (mm Hg)	100.4 ± 6.3	97.7 ± 6.7	98.5 ± 4.4
CaO_2 (ml/dl)	17.8 ± 1.9	17.7 ± 2.0	17.8 ± 2.0
MABP (mm Hg)	87 ± 14	86 ± 16	89 ± 14

Note. Values are means ± SD. CaO_2, total oxygen content of arterial blood; MABP, mean arterial blood pressure.

Data analysis

We analyzed 10 tomographic planes from 46.25 to 84.5 mm above and parallel to the orbitomeatal line, which corresponds to the levels from the basal ganglia and thalamus to the centrum semiovale. The region of interest (ROI) was placed on the CBF images during visual activation before acetazolamide administration. Based on the atlas prepared by Kretschmann and Weinrich (Kretschmann and Weinrich, 1986), the lower 3 images were examined by placing a total of 2 circular ROIs 16 mm in diameter compactly over the gray matter of the medial occipital cortex in each hemisphere along the posteroanterior direction from the posterior end (Fig. 1). These ROIs included the pericalcarine visual cortex and adjacent visual association cortex of the medial side. The same ROIs were transferred to the other 5 images. The mean value in the bilateral visual cortices was calculated as the average for all ROIs in both hemispheres. In addition, all 10 images were examined by placing a total of 10 to 12 circular ROIs 16 mm in diameter compactly over the gray matter of the outer cortex in each hemisphere (Yamauchi et al., 1990). The averaged values in all ROIs in the bilateral hemispheres, excluding the visual cortex ROIs, were referred to as the global values and were used for normalization of the values during visual stimulation to negate the effect of fluctuations in whole-brain values.

We corrected the CBF, $CMRO_2$, and $CMRO_2$/CBF values in the visual cortex during visual activation before and after acetazolamide administration by dividing by the correction factor of each variable: global values(visual activation)/global values(baseline). We calculated the percentage difference between the absolute or corrected values obtained during visual activation and the values at baseline (Δ%) as Δ% = [absolute or corrected CBF or $CMRO_2$ value (visual activation) - CBF or $CMRO_2$ value (baseline)]/[CBF or $CMRO_2$ value (baseline)] × 100 (%). Individual Δ% values were calculated and then averaged to give the mean percentage difference.

Statistical analysis

We compared the values of the PET variables obtained at baseline and during visual activation using the two-tailed paired *t* test. Linear regression analysis was used to analyze

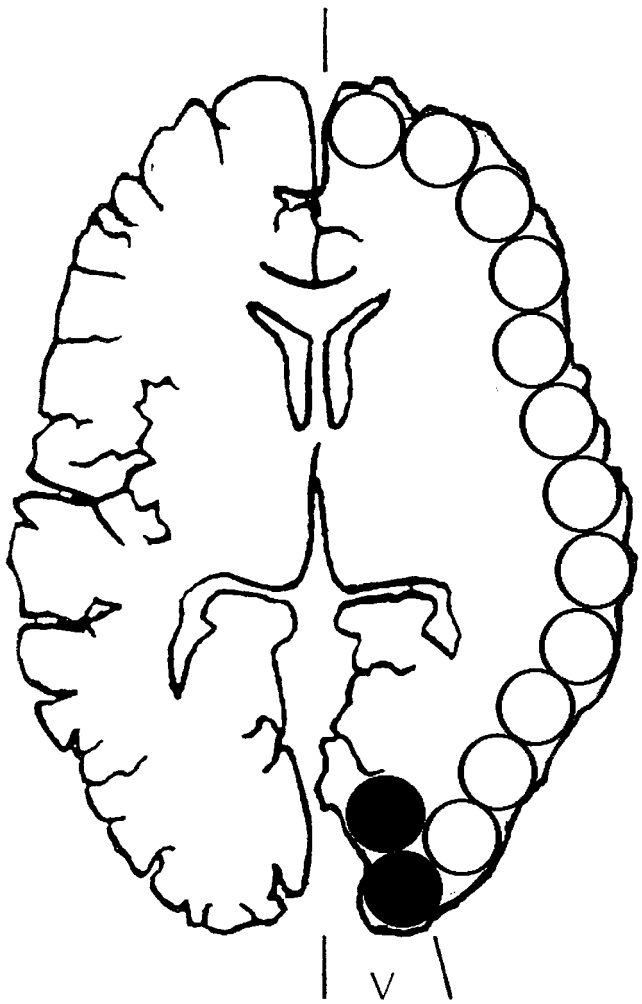

Fig. 1. A diagram illustrating the circular regions of interest placed over the cerebral cortex divided into the visual cortex (V, closed circles) and the gray matter of the outer cortex (open circles).

Table 2
Baseline and activation values of global CBF, $CMRO_2$, and $CMRO_2$/CBF

Variable	Baseline	Visual activation	
		Before administration of acetazolamide	After administration of acetazolamide
CBF (ml/100 g/min)	45.0 ± 5.5	43.2 ± 4.3	61.4 ± 7.6**
Correction factor		0.96 ± 0.04	1.37 ± 0.19#
$CMRO_2$ (ml/100 g/min)	3.94 ± 0.25	3.79 ± 0.38	3.64 ± 0.32*
Correction factor		0.95 ± 0.05	0.92 ± 0.07
$CMRO_2$/CBF (%)	8.82 ± 0.79	8.79 ± 0.81	6.03 ± 1.10**
Correction factor		0.99 ± 0.06	0.69 ± 0.16#

Note. Values are the mean ± SD. CBF, cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen.
* $P < 0.05$, ** $P < 0.01$ compared to baseline, # $P < 0.005$, compared to visual activation before administration of acetazolamide (paired *t* test).

the relationship between the values of the PET variables. A P value of <0.05 was regarded as indicating statistical significance.

Results

There were no significant differences among the global CBF, $CMRO_2$, and $CMRO_2$/CBF values during visual activation before acetazolamide administration and those at baseline, although a slight tendency for global CBF and $CMRO_2$ to decrease ($P = 0.09$ and 0.11, respectively) was observed (Table 2). During visual activation after acetazolamide administration, global CBF was significantly increased and global $CMRO_2$ and $CMRO_2$/CBF were significantly decreased, compared with those at baseline. The values of the correction factor [global values(visual activation)/global values(baseline)] for CBF and $CMRO_2$/CBF after acetazolamide administration were significantly different from those before, while the value of the correction factor for $CMRO_2$ was not.

The absolute CBF values in the visual cortex during visual activation both before and after acetazolamide administration were larger than those at baseline in all subjects, but the magnitude of the CBF change was increased after acetazolamide administration (Table 3). There was no significant difference between absolute $CMRO_2$ values during visual activation before acetazolamide administration and those at baseline, while $CMRO_2$ values during visual activation after acetazolamide administration were significantly decreased compared with baseline values. After acetazolamide administration, the magnitude of the $CMRO_2$ change was less than before in all subjects. Absolute $CMRO_2$/CBF values in the visual cortex during visual activation both before and after acetazolamide administration were lower than those at baseline in all subjects, but the magnitude of the change was augmented after acetazolamide administration.

The values of PET parameters in the visual cortex during visual activation after acetazolamide administration reflect the effects of both the visual activity and the acetazolamide. On the assumptions that a visual stimulus does not affect the rest of the brain and the effect of acetazolamide is the same throughout the cerebral cortical regions, the values during visual activation were corrected by the correction factor to isolate the visual activation-related changes in PET parameters by negating the effect of fluctuations in global values. The values of corrected CBF in the visual cortex during visual activation before and after acetazolamide administration were significantly different from those at baseline, but the magnitude of the CBF increase was reduced after acetazolamide administration (Table 4). No significant relationship was found between the CBF response during visual activation and the value of the correction factor for CBF

Table 3
Baseline and activation values of PET variables in the visual cortices

Variable	Baseline	Visual activation	
		Before administration of acetazolamide	After administration of acetazolamide
CBF (ml/100 g/min)	51.3 ± 5.8	68.0 ± 8.6*	84.0 ± 12.3*
% Change		33.7 ± 17.8	65.6 ± 30.2#
$CMRO_2$ (ml/100 g/min)	5.09 ± 0.34	5.06 ± 0.28	4.24 ± 0.35*
% Change		−0.45 ± 6.13	−16.5 ± 6.9#
$CMRO_2$/CBF (%)	9.99 ± 0.79	7.49 ± 0.63*	5.16 ± 1.08*
% Change		−24.8 ± 6.7	−48.1 ± 11.2#

Note. Values are the mean ± SD. CBF, cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen.
* $P < 0.005$ compared to baseline, # $P < 0.01$, compared to visual activation before administration of acetazolamide (paired *t* test).

Table 4
Baseline and corrected-activation values of PET variables in the visual cortices

Variable	Baseline	Visual activation	
		Before administration of acetazolamide	After administration of acetazolamide
CBF (ml/100 g/min)	51.3 ± 5.8	70.7 ± 9.0**	61.4 ± 7.5*
% Change		38.6 ± 15.0	20.3 ± 12.7#
$CMRO_2$ (ml/100 g/min)	5.09 ± 0.34	5.27 ± 0.13	4.59 ± 0.29*
% Change		3.99 ± 8.10	−9.61 ± 6.12#
$CMRO_2$/CBF (%)	9.99 ± 0.79	7.54 ± 0.90**	7.52 ± 0.59**
% Change		−24.3 ± 8.5	−24.4 ± 7.1

Note. Values are the mean ± SD. CBF, cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen. The values of CBF, $CMRO_2$, and $CMRO_2$/CBF during activation were corrected by dividing by the correction factor of each variable (global value(activation)/global value(baseline)).

* $P < 0.02$, ** $P < 0.005$ compared to the baseline, # $P < 0.005$, compared to visual activation before administration of acetazolamide (paired *t* test).

after acetazolamide administration ($P = 0.88$). After acetazolamide administration, corrected $CMRO_2$ was decreased in all subjects, and the magnitude of the $CMRO_2$ change was less than before, that is, a visual activation-related decrease in the $CMRO_2$ was consistently found. Although both the CBF and the $CMRO_2$ responses were decreased after acetazolamide administration, the corrected $CMRO_2$/CBF value during visual activation after acetazolamide administration was not different from that before, indicating that the relationship between $CMRO_2$ and CBF during visual activation was the same under both conditions.

Discussion

This study showed that acetazolamide decreased the responses of both CBF and $CMRO_2$ to visual stimulation. We found that in normal subjects, a 4-min visual stimulation induced a significant increase in CBF (33%) in the visual cortex compared with baseline values, but caused no significant change in $CMRO_2$, while no significant change in global CBF or $CMRO_2$ was observed. During visual stimulation after acetazolamide administration, both global and visual cortical CBF and $CMRO_2$ showed similar changes compared with the corresponding values at baseline (37 and 65% increases in CBF, and 8 and 16% decreases in $CMRO_2$, respectively). When visual cortical CBF and $CMRO_2$ values were corrected by the global values to isolate visual activation-related changes from acetazolamide-induced baseline shifts, the magnitudes of the visual cortical CBF and $CMRO_2$ responses after acetazolamide administration were lower than those before (20% vs 38% for CBF and −9 and 3% for $CMRO_2$). Carbonic anhydrase inhibitors, including acetazolamide, may decrease the efficacy of synaptic transmission by decreasing the extracellular and intracellular pH and are used as anti-epileptic drugs (Leniger et al., 2002). The effect of anti-epileptic drugs on neuronal excitation may be estimated by measuring the changes of the CBF and $CMRO_2$ responses during neural stimulation. The parallel decreases in the responses of CBF and $CMRO_2$ during visual stimulation after acetazolamide administration, as shown in this study, suggest a decrease in the magnitude of neuronal excitation and support the use of this drug as an anti-epileptic drug.

In this study, we also showed that the relationship between the visual activation-related responses in CBF and $CMRO_2$ changes after acetazolamide administration. After correction by the global values, a visual activation-related decrease in $CMRO_2$ together with an increase in CBF occurred during acetazolamide administration, as evaluated by the changes from baseline values. It has been proposed that total neuroenergetics, not changes in neuroenergetics, support localized brain activity during activation (Shulman et al., 1999; Hyder et al., 2002). The increase of CBF during visual stimulation seen in all the subjects studied suggested increased neuronal activity, and it is unlikely that a decrease in energy production caused neuronal activation. Therefore, the visual activation-related decrease in the $CMRO_2$ may have resulted from the sum of the changes of the energy metabolism in several compartments, e.g., neurons and glia, in the activated region (Dienel et al., 2002). Despite the reduced CBF response, the corrected $CMRO_2$/CBF values after acetazolamide administration were not different from those before, indicating that the relationship between the $CMRO_2$ and CBF values in response to visual activation is maintained with respect to the total neuroenergetics in the activated region both before and after acetazolamide administration. The decrease in the magnitude of neuronal excitation may cause a decrease of total neuroenergetics during visual activation, with constant corrected $CMRO_2$/CBF, after acetazolamide administration. The change of some metabolic components during visual activation may induce a decrease of total $CMRO_2$. The primary metabolic change associated with increases in neuronal activity may be an increase of nonoxidative glucose metabolism (Fox et al., 1988; Raichle, 1998), which may have occurred in the subjects with a decrease of $CMRO_2$ during neural stimulation in this study. A certain fraction of the oxidative metabolism of glucose occurring at baseline might change to nonoxidative metabolism upon stimulation, which may pro-

duce an efflux of lactate into the blood circulation without oxygen consumption (Shulman et al., 2001), leading to a decrease of $CMRO_2$.

A few studies have investigated the effect of acetazolamide on CBF and $CMRO_2$. An increase in global CBF and lack of change in global $CMRO_2$ were shown based on data about arteriovenous oxygen differences in the normal human brain (Posner and Plum, 1960; Vorstrup et al., 1984), while a significant decrease in $CMRO_2$ was reported based on a PET study of animals (Laux and Raichle, 1978). Our previous PET study with healthy subjects showed that acetazolamide induced a greater than 30% increase in global and regional values for CBF compared with baseline CBF values, but caused no significant change in $CMRO_2$, although a slight tendency for acetazolamide to reduce $CMRO_2$ was observed (Okazawa et al., 2001). In this study, during visual activation after acetazolamide administration, global CBF was significantly increased and global $CMRO_2$ was significantly decreased, compared with the corresponding values at baseline. The decrease in global $CMRO_2$ may have resulted from the combined effects of the visual activity and acetazolamide on global $CMRO_2$, because there was no significant difference between the values of the corrected factor before and after acetazolamide administration. Although the tendency for acetazolamide to decrease global $CMRO_2$ may be consistent with its antiepileptic effect, the effect of acetazolamide on global $CMRO_2$ appears to be small.

We could not measure CBF and $CMRO_2$ after acetazolamide administration in the visual stimulus off condition as the baseline condition for the visual stimulus on condition after acetazolamide administration. This was because the effect of acetazolamide on the CBF and $CMRO_2$ had probably decreased by 30 or 40 min after acetazolamide administration. Therefore, in the data analysis, we assumed that the effect of acetazolamide was the same throughout the whole brain and that the percentage difference between the corrected values obtained during visual stimulation after acetazolamide and the values at baseline (Δ%) reflected the visual stimulation-induced CBF or $CMRO_2$ response after acetazolamide administration. This is a reasonable assumption, because our previous investigation of the effect of acetazolamide on CBF and $CMRO_2$ in healthy subjects also showed that the effect of acetazolamide was similar throughout various cerebral cortical regions (Okazawa et al., 2001).

The decreased CBF response after the administration of acetazolamide may have resulted from acetazolamide-induced cerebral vasodilation. It might be speculated that mediators of neurovascular coupling were released, but resulted in only a small hemodynamic response due to acetazolamide-induced vasodilation. However, several previous studies using carbon dioxide as a vasodilator showed that cerebral vasodilation due to carbon dioxide does not decrease the neural activation-induced CBF response (Shimosegawa et al., 1995; Li et al., 1999). Acetazolamide induces vasodilation by a mechanism related to that of CO_2. In the steady state during sustained visual activation, carbon-dioxide or acetazolamide-induced vasodilation may not decrease the visual activation-induced CBF response, although it may slow the hemodynamic response after a brief visual stimulus (Bruhn et al., 1994; Cohen et al., 2002). The mechanism of vasodilation responsible for the visual activation-induced CBF change may be different from the mechanism of carbon-dioxide or acetazolamide-induced vasodilation. Different mediators or different sizes of arteries may contribute to vasodilation under these two conditions (Meyer and Gotoh, 1961; Iadecola et al., 1994). In the present study, the CBF response during visual stimulation was not correlated with the global CBF response, which indicates that there was no association between the decrease in CBF response and acetazolamide-induced vasodilation, on the assumption that the global CBF response reflects the degree of vasodilation caused by acetazolamide (Okazawa et al., 2001).

A previous study showed that the visual stimulation used in this study caused a 15% or greater change in peak pixel values of $CMRO_2$ after normalization of the whole brain $CMRO_2$ (Vafaee and Gjedde 2000). The difference of the level of $CMRO_2$ increase may have resulted from the pixel-by-pixel approach used in that study, compared with our ROI approach in this study.

The direct demonstration of the drug-induced changes in the CBF and $CMRO_2$ responses to neuronal activation shown in this study have some implications for fMRI studies, on the assumption that similar changes occur after other kinds of stimulation in other cortical areas. Most fMRI studies are based on the BOLD contrast caused by the alterations in the local deoxyhemoglobin content, which is affected by the changes in the CBF, $CMRO_2$, and CBV (Ogawa et al., 1990, 1993). A decrease in $CMRO_2$/CBF during activation is associated with an increased BOLD signal. The corrected $CMRO_2$/CBF values after acetazolamide administration were not different from those before, although the CBF response was decreased after acetazolamide administration, which may make the interpretation of the activation-related BOLD signal change difficult. Caution should be exercised when interpreting the change in the BOLD signal during neural activation as a quantitative index of the pharmacological effects on brain function.

In conclusion, although a strict interpretation of our results in terms of visual activation-related changes may be difficult, our results suggest that acetazolamide decreased the responses of both CBF and $CMRO_2$ during neural stimulation, which appears to support the idea that this drug affects neuronal excitability. Acetazolamide may modify the relationship between the visual activation-related changes in CBF and $CMRO_2$, as evaluated by the changes relative to baseline values. Therefore, if the BOLD response is to be used to measure drug-induced changes in neural activity, control experiments involving direct measurements of CBF and $CMRO_2$ responses will be necessary to dem-

onstrate that the drug does not change the relationship between neural activation-related changes in CBF and $CMRO_2$.

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