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CHANGES IN GLOBAL CEREBRAL BLOOD FLOW IN HUMANS: EFFECT ON REGIONAL CEREBRAL BLOOD FLOW DURING A NEURAL ACTIVATION TASK

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SUMMARY

1. The primary objective of this study was to examine in man, how induced changes in global cerebral blood flow (gCBF) affected a regional cerebral blood flow (rCBF) increase resulting from a neural activation task (opening of eyes). A secondary objective was to quantify how such induced changes in gCBF were distributed between representative regions of either predominantly grey matter or white matter.

2. Positron emission tomography with intravenous infusion of $H_2{}^{15}O$, was used to measure gCBF in six normal males. Concomitant measures of rCBF were obtained in three different regions of interest (ROI): a representative area of predominantly grey matter, a representative area of predominantly white matter and an area of visual cortex.

3. Cerebral blood flow was altered by establishing steady-state changes in P_{CO_2} at a near constant ventilation of approximately 30 l min^{-1}. The mean P_{ET,CO_2} ($\pm$ S.D.) levels (mmHg) that resulted were: low, $21{\cdot}8 \pm 1{\cdot}8$; normal, $39{\cdot}8 \pm 1{\cdot}0$, and high, $54{\cdot}8 \pm 1{\cdot}2$. The normal and high levels were obtained by adding appropriate amounts of CO_2 to the inspirate. The corresponding mean gCBF levels across all six subjects with eyes closed were: low, $24{\cdot}2 \pm 4{\cdot}6$; normal, $37{\cdot}2 \pm 3{\cdot}9$ and high, $66{\cdot}8 \pm 7{\cdot}6$ ml min^{-1} dl^{-1}.

4. Blood flow in grey matter (insular cortex) and white matter (centrum semiovale) at normal levels of P_{CO_2} averaged $56{\cdot}8 \pm 10{\cdot}1$ and $20{\cdot}3 \pm 3{\cdot}4$ ml min dl^{-1} respectively. As P_{CO_2} rose, the increase in rCBF to grey matter was approximately three times greater than that to white matter.

5. An activation state of eyes open in a brightly lit room was compared to a baseline state of eyes closed in a darkened room at the three levels of P_{CO_2} (and hence at three levels of gCBF). Over the whole gCBF range a significant ($P = 0{\cdot}028$) effect of increasing rCBF in the visual cortex ROI was found in response to opening

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the eyes; the effect of this activation on rCBF was not significantly dependent ($P = 0{\cdot}34$) on the P_{CO_2} (and hence gCBF) level. The effect of the activation on the rCBF was apparently 'additive' to the rise of rCBF associated with P_{CO_2}-related gCBF increase.

6. The results confirm the need to normalize for changes in gCBF during studies of rCBF in response to an activation protocol. They also provide support for the use of an 'additive' model to achieve such normalization provided that other cortical areas behave in a similar manner to that of the visual cortex.

INTRODUCTION

Regional blood flow in the brain is tightly coupled to local metabolic demand (Kuschinsky & Wahl, 1978; Siesjo, 1984) and hence is sensitive to regional neuronal activation; this was first shown in conscious man by Olesen (1971). Global cerebral blood flow (gCBF) does not, in general, reflect changes in regional cerebral blood flow (rCBF) unless the region activated is very large. The major factors that control gCBF are the perfusion pressure and the autoregulation mechanism, together with chemical and metabolic factors such as the P_{CO_2} in the capillaries, the H^+ ion concentration and P_{CO_2} in the perivascular space and the cerebral tissue P_{O_2} (Witzleb, 1987; Guyton, 1991).

To our knowledge, the dependency of an rCBF increase resulting from a neural activation upon the prevailing gCBF has not been studied previously. However, this has been noted as a problem by workers using positron emission tomography (PET) to study regional brain functions (Horowitz, Duara & Rapoport, 1984; Metter, Reige, Kuhl & Phelps, 1984; Ford, 1986; Moeller, Strother, Sidtis & Rottenberg, 1987). In the absence of experimental data and with the need to take account of within- and between-subject variation in gCBF (with the particular requirement to identify sites of cerebral activation using *intersubject* averaging), statistical models have been developed to normalize for the possible confounding effects of changes in gCBF. A proportional model (i.e. rCBF change is linearly dependent on gCBF and the relationship goes through zero) has been used by some workers (Fox, Mintun, Reimen & Raichle, 1988). Alternatively, an additive model has been proposed (Friston, Frith, Liddle, Dolan, Lammertsma & Frackowiak, 1990) based on the concept that changes in rCBF resulting from activation are independent of gCBF. In a study of neural activation resulting from differing verbal fluency tasks in four subjects, Friston *et al.* (1990) concluded that activated brain regions were more sensitive to global changes than would have been predicted by a simple proportional model and furthermore that the findings were consistent with an independence of regional and global change.

The present study therefore seeks to clarify the relationship between the prevailing level of gCBF and the increase in rCBF in specific sites resulting from local regional activation. We chose to change baseline gCBF over a wide range using controlled steady-state alterations in arterial P_{CO_2} (P_{a,CO_2}) at three levels. We designed our study such that breathing was kept constant. At each level we examined the rCBF response to a well-established activation protocol, visual cortical activation (opening of the eyes); this is known to produce a large increase in rCBF. During the course of these studies we also wished to compare the results of changing P_{a,CO_2} levels on the cerebral circulation and its distribution to

representative areas of grey and white matter, as elucidated by PET, with those obtained previously in animals and man using more invasive techniques.

Depending on the outcome of this study, we planned to further investigate whether hypercapnic stimulation of breathing activates any cerebral regions (Murphy, Meir, Adams & Guz, 1990) independently of the overall increase in gCBF, an inevitable consequence of an increase in P_{a,CO_2}.

METHODS

Subjects

Six normal right handed males (mean age 32; range 26–42 years) were studied; informed consent was obtained. The study was approved by the local ethical committee and approval to administer radioactive isotopes was given by the Administration of Radioactive Substance Approval Committee (ARSAC) UK. Two of the subjects were co-authors and the others were scientific colleagues.

Experimental protocols and measurements

Induction of changes in global cerebral blood flow (gCBF)

Changes in global cerebral blood flow in each individual were produced by establishing different levels of arterial P_{CO_2} at rest. To establish hypocapnia, subjects volitionally hyperventilated, with a constant respiratory rate of 10–12 breaths min^{-1} and a tidal volume of 1·5–2·0 l, to produce a stable end-tidal P_{CO_2} (P_{ET,CO_2}) of approximately 20 mmHg. To achieve normocapnia, this pattern of ventilation was maintained and dead space (corrugated plastic tubing; i.d. 3·5 cm) was added to establish a P_{ET,CO_2} of around 40 mmHg. Hypercapnia (P_{ET,CO_2} of around 55 mmHg) was established with the same ventilatory pattern but with additional dead space and supplementary inspired CO_2. In the presence of any dead space, normoxia was ensured by supplementary inspired oxygen sufficient to keep arterial oxygen saturation (finger probe oximetry; Ohmeda, Biox 3700e, Louisville, USA) at the subject's resting level (> 95 %). In all conditions, subjects breathed through a nasal mask connected to an ultrasonic flowmeter (Branta, Birmingham, UK) and CO_2 analyser (Hewlett Packard 47210A, USA).

Subjects required training to produce the 'standard breathing pattern' prior to PET scanning. Initially, they were provided with external cues by listening to a cycling ventilator, set at the desired respiratory frequency (f_R) and inspiratory time (T_I); the targeted tidal volume (V_T) was learnt by providing verbal feedback. Training was given over a number of sessions until subjects could maintain a constant breathing pattern for periods of 6 min without external cues and irrespective of different P_{CO_2} levels.

Visual activation protocol

The visual cortex was activated by the subjects keeping their eyes open with the room brightly lit; the control state was with the room darkened and the eyes closed.

PET scans

PET studies were performed using an ECAT 953B (CTI/Siemens, Knoxville, USA) dedicated head scanner; the performance characteristics have been described elsewhere (Spinks *et al.* 1992). The scanner was used in 2-dimensional mode with interplane tungsten septa in place (Townsend, 1991). Radiolabelled water ($H_2{}^{15}O$), produced continuously by the catalytic reaction of $^{15}O_2$ and hydrogen, was used as a tracer of cerebral blood flow. A venous line was inserted in the antecubital fossa on the left side to allow administration of the tracer. An arterial line was inserted into the left radial artery to allow monitoring of radiactive levels (for subsequent quantification of gCBF and rCBF values) and for analysis of blood gas levels. A polyurethane head mould was fitted to minimize head movement and the head was placed in the scanner so that the lowermost plane of scan acquisition was approximately parallel to and 15–18 mm above the orbito-meatal line. Subjects lay supine in the quiet darkened room with eyes closed while a transmission scan (using orbiting $^{68}Ge/^{68}Ga$ rods) was collected prior to tracer administration for the purpose of individual attenuation correction of emission data.

Cerebral blood flow studies were performed using a previously described method (Lammertsma *et al.* 1990; Colebatch *et al.* 1991). Briefly, between 1·8 and 2·9 GBq of $H_2{}^{15}O$ was administered intravenously at a constant rate infusion of 10 ml min^{-1} over 1 min; this was followed by a 30 s

saline flush. A multi-frame dynamic scan was acquired over a 4 min period starting 30 s prior to the start of the intravenous infusion. Arterial levels of radioactivity were monitored continuously using an on-line detection system as described previously (Colebatch *et al.* 1991). Global and regional cerebral blood flow could then be calculated over the period of each scan.

Protocol

Six scans were collected for each subject with periods of at least 10–12 min between scans to allow for both decay of radioactivity and any required re-establishment of normocapnia from the previous measurement. Two scans were performed at each level of P_{ET,CO_2}; one of each pair was in the rest condition with eyes closed while the other was in the neural activation condition of eyes open. The ordering of conditions was balanced within a study and across subjects. The stable ventilatory pattern and the P_{ET,CO_2} required were established for at least 2·5 min before the beginning of the scan and then maintained during the scanning period. A 2 ml sample of arterial blood was taken immediately at the end of the scanning period for analysis of pH, P_{CO_2} and P_{O_2} (Novastat Profile 5, Nova Biomedical, Waltham, MA, USA).

Blood pressure measurement

On a separate occasion, blood pressure was measured (Sphygmomanometry with Korotkov sounds) during identical experimental conditions, but without PET scanning, in five of the six subjects.

Image analysis

Image analysis was performed on a SPARC1 computer (Sun Microsystems Europe Inc., Surrey, UK) using an interactive image analysis software package (ANALYZE, Biodynamic Research Unit, Mayo Clinic, Rochester, MN, USA). Calculations and image matrix manipulations relating to the identification of sites of activation were performed in PRO-MATLAB (The Mathworks Inc., Sherbon, MA, USA). To increase the validity and precision of the 'regions of interest' (ROI) placement, all the images were stereotactically normalized as previously described (Friston, Frith, Liddle & Frackowiak, 1991). Following normalization, the data correspond to the standard brain dimensions used by Talairach & Tournoux (1988) in their stereotactic atlas. The resulting images consisted of twenty-six planes of voxels (i.e. slices) measuring 2 mm by 2 mm by 4 mm in the x (right and left of midline), y (rostral and caudal to the anterior commissural line) and z (dorsal and ventral to the intercommissural plane) directions respectively.

Measurement of global cerebral blood flow

A whole brain ROI was drawn directly on the raw data of the dynamic images; the scalp was excluded. This region was drawn in the middle third of the brain and global flows were then calculated using the technique of Lammertsma *et al.* (1990).

Identification of regions of interest and calculation of their rCBF

Without activation – grey and white matter. Grey and white matter ROIs were chosen from one of the cerebral hemispheres. The grey matter ROI was sited in the insula and its operculae from the frontal and temporal lobes (see inset of Fig. 2); this region was positioned on a number of planes (3–6) which varied between subjects to allow the same gyral anatomy to be included from brains of different size. The volume of brain identified was 10–15 ml with the central plane 4 mm below the intercommissural line. The white matter ROI was sited in the centrum semiovale region (see inset of Fig. 2) using three planes; the volume of brain identified was approximately 5 ml with the central plane 28 mm above the intercommissural line.

To obtain the highest possible degree of accuracy, these ROIs were projected on the original dynamic frames in order to obtain grey and white matter time–activity curves. These curves were then fitted to give both rCBF and the volume of distribution of water, taking into account delay and dispersion of the arterial input function as described previously (Lammertsma *et al.* 1990); this allowed the computation of absolute blood flow.

With visual activation task. The sites of neuronal activation with these tasks were defined during normocapnia using the functional CBF data from all subjects (obtained with a fixed volume of distribution of water of 0·95) as described previously (Lammertsma *et al.* 1990); the anatomical variation between subjects was reduced by convolving the data with a Gaussian filter (full-width half-maximum 20 mm). Any physiological variation resulting from differences in gCBF was corrected for by treating global counts as a confounding variable using an analysis of covariance (ANCOVA; Friston *et al.* 1990). Adjusted condition means and variances were

compared using linear contrasts and the resultant three dimensional maps of t statistical values (corrected for multiple non-independent comparisons) for the $P < 0.05$ level of significance were then displayed (Friston *et al.* 1991). The visual activation task produced large rCBF increases bilaterally in the regions around and just superior to the calcarine fissure (see Results), and ROIs were defined on the three planes with the highest values. On each of these planes the voxel with the highest t value was defined as the centre of a circular region with a volume of thirty-two voxels (i.e. each region approximately 0·5 ml). These regions were then applied to anatomically normalized unsmoothed images which had not been subjected to an ANCOVA correction for global flow. The mean voxel value was then determined for these regions and this was used to determine the rCBF within the visual ROI for each subject in each of the six experimental scans.

Statistical analysis

To test the constancy of breathing at different levels of P_{ET,CO_2} and with visual neural activation, mean values (over each scan) of inspired minute ventilation ($\dot{V}_I$), V_T, f_R and P_{ET,CO_2} were compared between conditions using a two-factor analysis of variance (BMDP) with CO_2 level (low:normal:high) and activation state (rest:activation) as factors. Differences in rCBF between grey and white matter and the dependency of any such differences on the level of gCBF were examined using a two-factor analysis of variance with CO_2 level (low:normal:high) and anatomical site (grey:white) as factors. Differences in gCBF and in rCBF in response to visual neural activation and the dependence of any differences on the level of gCBF were examined using a two-factor analysis of variance with CO_2 level (low:normal:high) and activation state (rest:activation) as factors; for all comparisons $P < 0.05$ in a two-tailed test was taken as indicating a statistically significant difference. The least significant difference of Fisher (1935) was calculated from the analysis of variance to show the smallest difference between any two means which is statistically significant.

RESULTS

Subjects' comments

The subjects confirmed that during the scans they had remained awake and had kept their eyes open or closed as requested. All subjects were confident that they had been able to maintain a fairly uniform pattern of breathing during the tasks although they all commented that this had been more difficult in the hypercapnic condition due to an uncomfortable feeling of needing to breathe more. Subjects also reported feeling hot and flushed during hypercapnia; by contrast most felt cool or even cold during hypocapnia. There were no reports of any other symptoms during hypocapnia and there was no evidence of tetany. Subjects did not report any difference in the attention required to execute the breathing task either at different CO_2 levels or during neural activation runs.

Pattern of breathing

Individual values for breathing pattern and end-tidal P_{CO_2} (P_{ET,CO_2}), at the three levels of CO_2 and during the rest and activation states are shown in Fig. 1. The results of the analysis of variance indicated the expected statistically significant differences in mean P_{ET,CO_2} levels between hypocapnia (21·0 mmHg), normocapnia (40·0 mmHg) and hypercapnia (54·6 mmHg). Mean $\dot{V}_I$ was significantly greater at the high CO_2 level (mean = 34·9 l min^{-1}) compared with the low (29·8 l min^{-1}) and normal (29·7 l min^{-1}) levels although there were no significant differences for mean f_R (low, 16·9; normal, 16·9; high, 18·5 breaths min^{-1}) or V_T (low, 1·76; normal, 1·74; high, 1·84 l). Comparison between rest and activation states over the range of CO_2 levels showed that mean f_R was significantly greater with activation (18·2 min^{-1}) compared with rest (16·7 min^{-1}); there were no significant differences for P_{ETCO_2}, $\dot{V}_I$, or V_T.

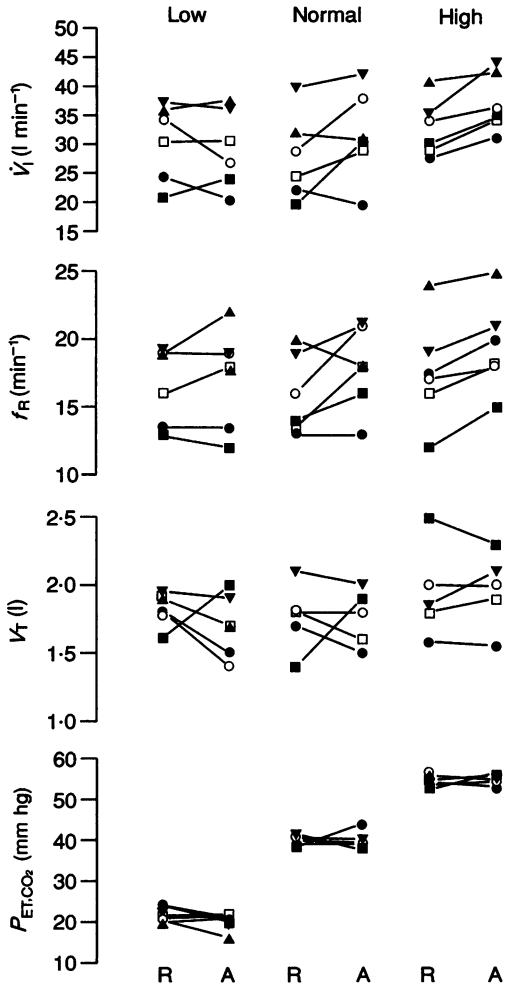

Fig. 1. Average values (over 1 min of $H_2{}^{15}O$ infusion) of inspired minute ventilation $\dot{V}_I$, respiratory frequency (f_R), tidal volume (V_T) and end-tidal P_{CO_2} (P_{ET,CO_2}) for each of 6 subjects (●, subject 1; ▼, subject 2; ■, subject 3; ▲, subject 4; ○, subject 5; □, subject 6) in each of the experimental conditions. Low, normal and high represent the three P_{CO_2} levels at which the scans were performed during resting (R) and visual activation (A) conditions. Note the close matching of ventilatory variables across the experimental conditions and between rest and activation states despite clear differences in P_{ET,CO_2}.

Blood pressure and arterial blood gas levels

In five subjects, tested at a different time, the average blood pressures during the steady-state periods of P_{CO_2} were: 120/75 during hypocapnia, 122/78 during normocapnia and 143/86 mmHg during hypercapnia. Analysis of variance showed that the mean blood pressure (± S.D.) was significantly higher ($P = 0{\cdot}018$) during hypercapnia (105 ± 10) compared with hypocapnia (90 ± 8) or normocapnia (92 ± 8 mm Hg). In general, there was close agreement in individuals between

arterial P_{CO_2} measured at the completion of a scan and end-tidal P_{CO_2} averaged over the period of scanning ($\pm$ 2 mmHg); however in a few instances there were discrepancies between the two measurements ($\pm$ 5 mmHg). In view of this, P_{ET,CO_2} measurements were utilised in subsequent analyses since they relate better to the period of scanning and do not depend on a single measurement. The average arterial P_{O_2} measurements (rest and activation runs combined) were: 128 ± 11 during hypocapnia; 118 ± 16 during normocapnia and 129 ± 15 mmHg during hypercapnia. Analysis of variance showed that there were no statistically significant differences between these levels ($P = 0{\cdot}158$).

Effect of P_{CO_2} on global cerebral blood flow

The values for P_{ET,CO_2} and corresponding measurements of gCBF at rest with eyes closed are given for each subject in Table 1. The mean gCBF during normocapnia was $37{\cdot}2 \pm 3{\cdot}9$; during hypocapnia it was $24{\cdot}2 \pm 4{\cdot}6$; and during hypercapnia it was $66{\cdot}8 \pm 7{\cdot}6$ ml min^{-1} dl^{-1}. The average ratio (from individual values) of ΔgCBF:ΔP_{CO_2} for the low to normal P_{CO_2} change was $0{\cdot}72 \pm 0{\cdot}15$ (range 0·44–0·85); and for the normal to high P_{CO_2} change it was $2{\cdot}00 \pm 0{\cdot}32$ (range 1·40–2·26) ml min^{-1} dl^{-1} mmHg^{-1}.

Effect of P_{CO_2} on blood flow in grey and white matter

For each individual, the measurement of gCBF obtained in each of the three scans (i.e. low, normal and high P_{CO_2}) in the rest (eyes closed) condition was paired with the rCBF measurement for the grey matter region of interest (ROI) and separately with the rCBF for the white matter ROI. These data have been plotted for each of the six subjects in Fig. 2. The mean values with normocapnia for grey and white matter were $56{\cdot}8 \pm 10{\cdot}1$ and $20{\cdot}3 \pm 3{\cdot}4$ ml min^{-1} dl^{-1} respectively. In each subject, when related to P_{ET,CO_2} (Fig. 2*A*), the increase in rCBF for grey matter is greater than for white matter, particularly in the normal to high P_{CO_2} range. Thus in the low to normal P_{CO_2} range, the mean changes in blood flow for grey and white matter are $0{\cdot}99 \pm 0{\cdot}60$ and $0{\cdot}39 \pm 0{\cdot}14$ ml min^{-1} dl^{-1} mmHg^{-1} respectively; these changes are significantly different (paired t test; $P = 0{\cdot}01$). For the normal to high P_{CO_2} range, the mean change in blood flow for grey matter is $2{\cdot}97 \pm 1{\cdot}07$ and for white matter this ratio is $1{\cdot}10 \pm 0{\cdot}34$ ml min^{-1} dl^{-1} mmHg^{-1}; these ratios are significantly different (paired t test; $P = 0{\cdot}001$). Figure 2*B* shows the same grey and white matter rCBF values for each subject, but in this case plotted against the prevailing gCBF value at each of the P_{CO_2} levels. For each subject there is a nearly linear increase in rCBF as gCBF rises, with intercepts close to zero. The mean ratio over the entire range of gCBF (i.e. ΔrCBF/ΔgCBF) is $1{\cdot}53 \pm 0{\cdot}24$ for grey and $0{\cdot}52 \pm 0{\cdot}18$ for white matter.

Analysis of variance on the pooled data confirms the obvious effect on rCBF for both grey and white matter ROIs when the gCBF is increased by P_{CO_2} ($P < 0{\cdot}0001$); furthermore the rCBF within grey matter was significantly different from that within white matter over the range of P_{CO_2} ($P < 0{\cdot}0001$). The presence of a significant interaction term ($P < 0{\cdot}0001$) between the P_{CO_2} factor and the ROI factor confirms that the pattern of increase in rCBF within grey matter was significantly different from that within white matter as gCBF increased.

Effect of visual activation on rCBF

Opening the eyes activated the primary visual cortex bilaterally (see Methods); the three dimensional map of the t statistical values at normocapnia showed significance at the $P < 0{\cdot}05$ level. The rCBF associated with visual activation was determined from the mean voxel values in the visual ROIs (see Methods). These

TABLE 1. Individual and mean values for end-tidal P_{CO_2} (in mmHg), regional cerebral blood flow (rCBF; in ml min^{-1} dl^{-1}) in a visual region of interest and global cerebral blood flow (gCBF; in ml min^{-1} dl^{-1}).

		Condition					
Subject number		Low P_{CO_2}		Normal P_{CO_2}		High P_{CO_2}	
		Eyes closed	Open	Eyes closed	Open	Eyes closed	Open
1	P_{CO_2}	24	21	39	44	55	53
	gCBF	29	28	39	48	72	68
	rCBF	32	37	41	67	84	79
2	P_{CO_2}	22	21	39	40	55	54
	gCBF	23	23	37	39	68	72
	rCBF	27	26	37	40	76	74
3	P_{CO_2}	24	20	41	38	53	56
	gCBF	18	19	31	32	58	57
	rCBF	38*	26	32	38	62	70
4	P_{CO_2}	20	16	40	40	56	55
	gCBF	26	26	43	45	75	74
	rCBF	27	34	48	57	78	87
5	P_{CO_2}	20	21	41	39	56	54
	gCBF	20	21	36	31	57	60
	rGBF	27	33	41	42	66	73
6	P_{CO_2}	21	22	39	40	54	54
	gCBF	29	29	37	38	71	76
	rCBF	37	40	44	57	80	89
Mean	P_{CO_2}	21·8	20·2	39·8	40·3	54·8	54·4
	gCBF	24·2	24·3	37·2	38·3	66·8	67·8
	rCBF	31·4	32·7	40·5	50·1	74·2	78·7

Values are given for each of the three P_{CO_2} conditions (low, normal and high) and for the rest (eyes closed) and activation (eyes open) states. * indicates a presumed invalid measurement due to undefined error (see text).

values are given for each condition for each subject, together with the gCBF and P_{ET,CO_2}, in Table 1; the mean values for P_{ET,CO_2}, gCBF and rCBF are also given. Examination of the data shows one point (subject 3, low P_{CO_2}, eyes closed) with a high rCBF which reduces markedly in response to a visual activation; it was thought likely that this point was a result of undefined experimental error. Figure 3 presents the mean data of the group of five subjects (i.e. excluding subject 3) of the effect of visual activation on rCBF at the different gCBFs resulting from changes in P_{CO_2}. Analysis of variance on the data from these five subjects shows an overall (i.e. across the range of P_{CO_2} studied) significant effect of visual activation

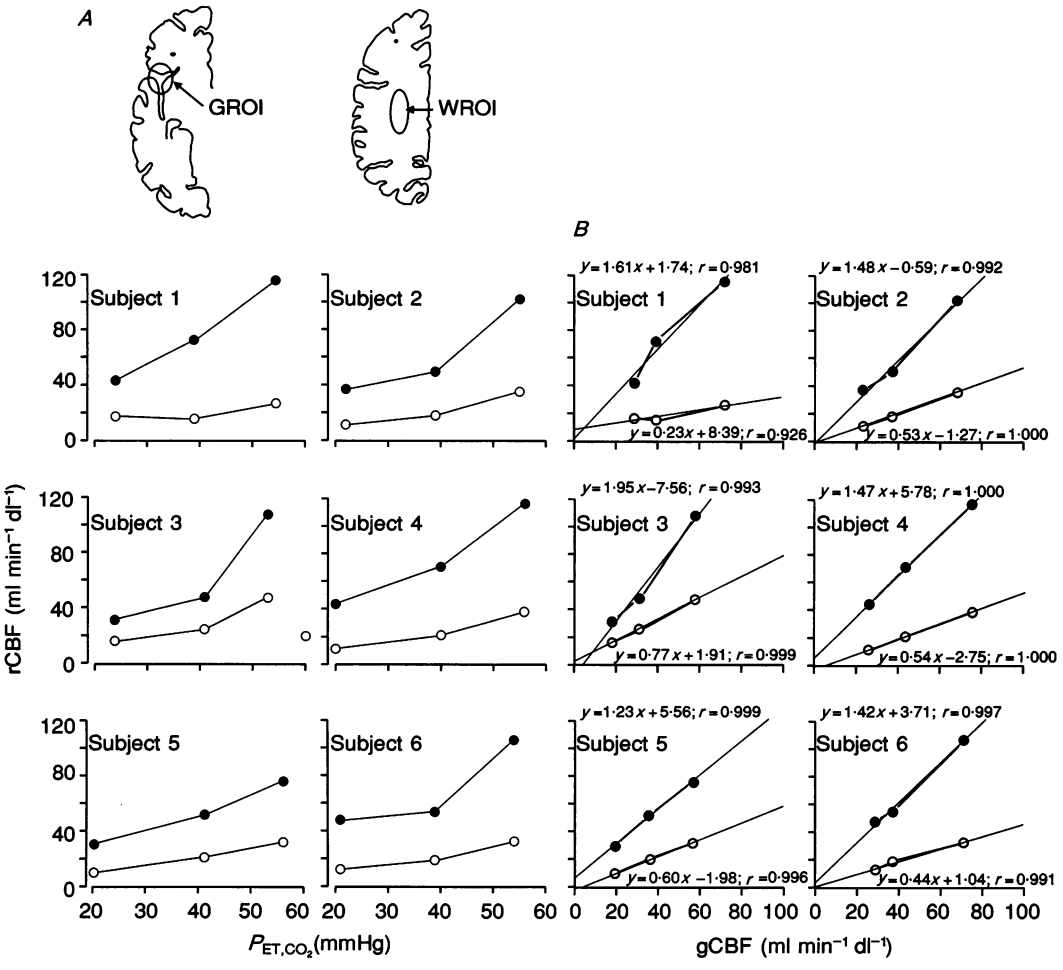

Fig. 2. Values of regional cerebral blood flow (rCBF) in standard cerebral regions comprising predominantly grey matter (●) and white matter (○) for each of six subjects in the absence of visual activation (eyes closed). The inset shows diagramatically the areas chosen for the grey matter region of interest (GROI; the insula and its operculae from the frontal and temporal lobes) and the white matter region of interest (WROI; the centrum semiovale region). The same rCBF data are plotted separately against the end-tidal P_{CO_2} (P_{ET,CO_2}; Fig. 2*A*) and global cerebral blood flow (gCBF; Fig. 2*B*). With respect to P_{ET,CO_2} the changes in rCBF are greater for grey matter than for white matter particularly with hypercapnia. With respect to gCBF, rCBF in each subject increased linearly for both grey and white matter; for each subject, regression equations have intercepts close to the origin but slopes are invariably greater for grey matter. y is rCBF, x is gCBF and r is the correlation coefficient.

($P = 0{\cdot}028$) on rCBF; the same activation produces a minor, non-significant ($P = 0{\cdot}083$) increase in gCBF. There was no significant interaction term between the P_{CO_2} factor and the activation factor ($P = 0{\cdot}340$) on rCBF implying that the effect of activation on rCBF was not dependent on the P_{CO_2} level and hence on gCBF. Similarly, there was no significant interaction term between the P_{CO_2} factor and the activation factor ($P = 0{\cdot}787$) on gCBF.

Analysis of variance on the data from all six subjects (i.e. including subject 3) still shows an overall significant increase in rCBF of visual activation ($P = 0{\cdot}031$) and a non-significant interaction term between the P_{CO_2} factor and the activation factor ($P = 0{\cdot}233$) on rCBF. The only apparent effect of including the data from subject 3 was to minimize the difference in rCBF due to visual activation at a low P_{CO_2} (Table 1, cf. Fig. 3).

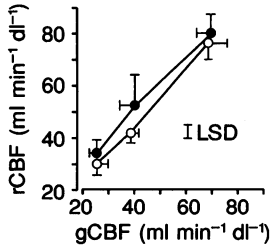

Fig. 3. Mean values (±S.D. bars) of regional cerebral blood flow (rCBF) in the visual cortical ROI with eyes closed (○) and eyes open (●) plotted against global cerebral blood flow (gCBF) resulting from the change in P_{CO_2} in 5 of the 6 subjects (see text). Fisher's (1935) least significant difference (LSD) is presented geometrically to indicate the difference between rCBF values necessary to achieve statistical significance at $P < 0{\cdot}05$ (see text). At each P_{CO_2} level, visual activation (opening eyes) is associated with greater increases in rCBF compared with the change in gCBF.

DISCUSSION

The most crucial finding in the present study is the demonstration that neural activation of the visual cortex causes an increase in regional cerebral blood flow (rCBF) which is independent of the prevailing level of global cerebral blood flow (gCBF). It has considerable practical relevance for the normalization of rCBF measurements to take account of variations in gCBF which occur during neural activation studies. A further significant aspect of the present study is that we have been able to use PET to confirm and extend previous observations in man (using 'inert gas'techniques) on the effect of increased arterial P_{CO_2} on CBF and its relative distribution to representative areas of grey and white matter.

We have documented a mean range in gCBF from 24–67 ml min^{-1} dl^{-1} as end-tidal P_{CO_2} was experimentally adjusted between 22 and 55 mmHg. By achieving this at a relatively constant ventilation for each P_{CO_2} level, we were able to control for any possible confounding influence that the act of breathing itself might have on gCBF. The average level of gCBF recorded in the present study under resting normocapnic conditions was 37 ml min^{-1} dl^{-1}. This is somewhat lower than the range of 45–55 ml min^{-1} dl^{-1} identified by Lassen (1985) as the 'Gold Standard' in a critical review of the literature. The fact that we obtained a value for gCBF below this range, strongly suggests a systematic error probably arising in the present study from the choice of the middle third of the brain (see Methods) to obtain data for subsequent normalization by a geometrical estimate of *total* intracranial volume; this would include the ventricles and cisternae even though radioactive 'counts' relate essentially to the vascularized regions.

In the present study, we have confirmed previous observations in man that P_{CO_2} alterations cause substantially greater changes in CBF above the normocapnic level

than in the hypocapnic range (see Fig. 2). Between 40 and 55 mmHg P_{CO_2}, the mean $\Delta gCBF/\Delta P_{CO_2}$ in the present study was 2·0 ml min^{-1} dl^{-1} $mmHg^{-1}$; between 40 and 21 mmHg P_{CO_2} the mean $\Delta gCBF/\Delta P_{CO_2}$ was 0·7 ml min^{-1} dl^{-1} $mmHg^{-1}$; these values are similar to those derived using inert gas techniques (Kety & Schmidt, 1948*a*; Novack, Shenkin, Bortin, Guloboff & Soffe, 1953). Thus the present findings, taken together with the previous observations cited, lend no support to the view that it is permissible to calculate the slope of the best fitting straight line through gCBF data in the range of P_{CO_2} between 20–60 mmHg (Reivich, 1964), or to use such a slope to normalize values of gCBF to a P_{CO_2} of 40 mmHg as suggested by Purves (1972).

The average rise in mean arterial blood pressure with hypercapnia in the present study of 13 mmHg for an average elevation of P_{CO_2} of 15 mmHg compares well with previous observations (Kety & Schmidt, 1948*b*; Novak *et al.* 1953). During normocapnia, gCBF in man is independent of arterial blood pressure (Lassen, 1959). However, with cerebrovascular dilatation consequent upon the rise in P_{CO_2}, such autoregulation may well be impaired and if this is so, the small elevations in blood pressure documented with hypercapnia may contribute to the increasing $\Delta CBF/\Delta P_{CO_2}$ in the hypercapnic compared to the hypocapnic range.

Since CBF is insensitive to changes in arterial P_{O_2} above 60–80 mmHg (Lambertsen, 1961; Borgstrom, Johannson & Siesjo, 1975) it is unlikely that the small range of mean arterial P_{O_2} noted between the different conditions of this study (i.e. 118–129 mmHg) could have had any significant influence on the CBF levels recorded.

The mean values for grey and white matter rCBF found with normocapnia were similar to those reported by Lammertsma *et al.* (1990) using a similar technique. The present study indicates a disproportionate increase in rCBF in grey compared with white matter as P_{CO_2} rises above the normocapnic range suggesting that the cerebrovascular resistance falls much more in grey than in white matter under these conditions. The reason for this phenomenon is unclear but it has been reported previously in anaesthetized animals (Hansen, Sultzer, Freygang & Sokoloff, 1957; James, Millar & Purves, 1969).

The nature of the relationship between focal CBF changes associated with activation and levels of global CBF is unclear. Since many activation protocols induce only relatively small changes in rCBF compared to the absolute value of gCBF, interpretation of such studies requires that changes in gCBF be carefully considered. Clearly, if there is a non-specific change in gCBF, it is important to be sure that any rCBF change in a ROI is not a mere reflection of the altered gCBF. Changes in gCBF could occur in a single subject over time, and also between individual subjects, as well as resulting from factors, such as exercise, anxiety or changes in acid–base status (as in the present study). In such cases, one would need to normalize for changes in gCBF in order that small changes in rCBF due to activation could be more readily identified. On the other hand, it is possible that the activation task itself may significantly alter global CBF directly through large focal changes; normalization for differences in gCBF in this case might be expected to downgrade or eliminate the effect in which one was interested (Chadwick & Whelan, 1991).

A number of methods have been proposed to take account of global, region-independent, inter- and intra-subject variation during regional neural activation

studies (Horowitz *et al.* 1984; Metter *et al.* 1984; Moeller *et al.* 1987). Fox *et al.* (1988) have proposed a normalization method for gCBF variation which simply involves dividing rCBF by the estimated average gCBF; this proportional model necessarily assumes a relationship between rCBF and gCBF which is both linear and passes through the origin. Friston *et al.* (1990) have compared this proportional model with an additive model in which neural activation in a ROI would result in a similar increase in rCBF over a range of gCBF and have concluded, using a physiological activation protocol (verbal fluency task) that their additive model fits the observed data better than the proportional model. Their conclusion was arrived at through the use of an analysis of covariance which treats gCBF as a confounding 'nuisance' covariate which combines with the 'effect of interest' (rCBF changes solely due to neural activation) to produce variance in what is measured (rCBF changes in the ROI). However, their conclusion may be limited by the relatively narrow variations in gCBF which is likely to have accompanied their activation task.

The present study permits a more direct analysis of the inter-relationships between global and regional blood flow changes encountered during PET studies. By inducing changes in gCBF over a wide physiological range it was possible to examine the effects on rCBF, firstly, in representative areas of grey and white matter in the absence of activation and secondly, in response to a well-defined visual activation task. In this way, the appropriateness of normalization, based on clear-cut changes in gCBF could be judged. Our study confirms that across a wide range of gCBF, the relationship between gCBF and rCBF without activation is well described by a simple linear model for the defined areas of grey and white matter (see Fig. 2*B*) and also in the visual cortex (Fig. 3). Moreover, this study highlights the fact that the ratio of ΔrCBF/ΔgCBF is much higher for grey matter and clearly demonstrates the need for normalization of even small non-specific changes in gCBF during activation studies. With respect to changes in gCBF in response to visual activation, the present study indicates that at a normal arterial P_{CO_2}, there is a 23 % increase in rCBF in a visual cortical ROI in the face of only a 3 % non-significant increase in gCBF (Table 1; Fig. 3). Hence, with this activation, any normalization for changes in gCBF (which may have been induced by the activation) would not have greatly limited the ability to identify an increase in rCBF in response to neuronal activation. A different activation, producing much smaller increases in rCBF with a similar increase in gCBF would not have been so easily detected. Since in practice, it is impossible to know to what extent any changes in gCBF reflect non-specific as opposed to activation-related mechanisms, the present results point to the fact that normalization for changes in gCBF is indeed necessary.

The present study demonstrates that the increase in rCBF in the visual cortex, which accompanies opening of the eyes, shows no systematic dependence on the level of gCBF over a wide physiological range. Thus, although the increase in rCBF in response to visual activation was apparently greatest at a normal arterial P_{CO_2}, there were significant increases in rCBF associated with the same visual activation at both low and high levels of P_{CO_2} and hence gCBF (Fig. 3). One might hypothesize that the activation-related increase in rCBF at low P_{CO_2} is reduced because of the intense prevailing vasoconstriction, while at a high P_{CO_2} there is such an excess of

blood flow that the degree of vasodilatation present may be approaching a maximum. In the range of P_{CO_2} studied, and allowing for the relatively small data set, both the presentation of the results shown in Fig. 3 and the non-significant interaction term ($P = 0{\cdot}340$) between P_{CO_2} (gCBF) factor and the activation (rCBF) factor does not provide any basis for rejecting the hypothesis that the plots of rCBF against gCBF with eyes closed or opened are parallel to each other. Hence there is no support for a proportional relationship between rCBF increases with activation and the prevailing level of gCBF. The data support an approximation to an additive model as described by Friston *et al.* (1990) but it must be acknowledged that our limited data set do not allow us to reject the proportional hypothesis.

The results from the present study have a practical significance to those workers measuring rCBF to investigate activation of regional cerebral areas under conditions where global cerebral blood flow could change in a systematic way. Any change in gCBF would necessarily be associated with non-specific changes in rCBF in a region of interest. However, the fact that a more specific neural activation was still detectable over such a wide range of gCBF implies that normalization, on the basis of an 'additive model', should not limit the ability to study focal changes in the face of even substantial changes in total blood flow. If our results with respect to visual activation are representative of other cortical areas, then studies on areas in the brain activated by CO_2 inhalation (e.g. any cerebral areas associated with respiratory control) should be possible.

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