Baroreflex-induced sympathetic activation does not alter cerebrovascular CO$_2$ responsiveness in humans

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We investigated the effect of baroreflex-induced sympathetic activation, produced by lower body negative pressure (LBNP) at −40 mmHg, on cerebrovascular responsiveness to hyper- and hypocapnia in healthy humans. Transcranial Doppler ultrasound was used to measure blood flow velocity (CFV) in the middle cerebral artery during variations in end-tidal carbon dioxide pressure (P_{ET,CO_2}) of +10, +5, 0, −5, and −10 mmHg relative to eupnoea. The slopes of the linear relationships between P_{ET,CO_2} and CFV were computed separately for hyper- and hypocapnia during the LBNP and no-LBNP conditions. LBNP decreased pulse pressure, but did not change mean arterial pressure. LBNP evoked an increase in ventilation that resulted in a 9 ± 2 mmHg decrease in P_{ET,CO_2}, which was corrected by CO$_2$ supplementation of the inspired air. LBNP did not affect cerebrovascular CO$_2$ response slopes during steady-state hypercapnia (3.14 ± 0.24 *vs.* 2.96 ± 0.26 cm s^{-1} mmHg^{-1}) or hypocapnia (1.31 ± 0.18 *vs.* 1.32 ± 0.19 cm s^{-1} mmHg^{-1}), or the CFV responses to voluntary apnoea ($+51 \pm 19$ *vs.* $+50 \pm 18$ %). Thus, cerebrovascular CO$_2$ responsiveness was not altered by baroreflex-induced sympathetic activation. Our data challenge the concept that sympathetic activation restrains cerebrovascular responses to alterations in CO$_2$ pressure.

The extraparenchymal cerebral arteries, down to and including the pial arteries, are richly innervated by sympathetic nerve fibres (Nielsen & Owman, 1967; Falck, *et al.* 1968; Nelson & Rennels, 1970; Edvinsson, 1975). Previous investigators have shown that electrical stimulation of the superior cervical ganglion, the stellate ganglion, or the cervical sympathetic trunk can elicit substantial reductions in cerebral blood flow in cortical and brainstem regions (Meyer *et al.* 1967; James *et al.* 1969; Harper *et al.* 1972; Aubineau *et al.* 1975). This experimental evidence notwithstanding, the traditional thinking is that, under physiological conditions, the role of the sympathetic nervous system in control of cerebral blood flow is limited mainly to modulation of the cerebrovascular responses to alterations in arterial CO$_2$ pressure P_{a,CO_2} (James *et al.* 1969; Kobayashi *et al.* 1971; Harper *et al.* 1972; Wei *et al.* 1980).

Previous investigators have hypothesized that activation of the sympathetic nervous system produces cerebral vasoconstriction during orthostatic stress in humans (Giller *et al.* 1992; Levine *et al.* 1994). Augmented sympathetic outflow to the cerebral circulation is a putative cause of decreased cerebral blood flow and lightheadedness in

individuals with idiopathic orthostatic intolerance (Jordan *et al.* 1998); however, this sign and symptom have also been linked to the hyperventilation-induced hypocapnia that often occurs when such patients assume the upright posture (Lagi *et al.* 2001). In healthy humans, cerebrovascular responses to experimental manipulations of CO$_2$ tension were observed to be diminished during head-up tilt and augmented during ganglionic blockade, effects the authors attributed to sympathetic activation and deactivation, respectively (Jordan *et al.* 2000). In a previous study from our laboratory, we used a comparable ganglionic blockade protocol but did not replicate the finding that removal of basal sympathetic tone augmented cerebrovascular responses to hyper- and hypocapnia (Przybylowski *et al.* 2003). The purpose of the present study, therefore, was to test the hypothesis that augmentation of sympathetic vasoconstrictor outflow diminishes cerebrovascular CO$_2$ responsiveness. Accordingly, in healthy subjects, we measured cerebral blood flow velocity (CFV) during manipulations of CO$_2$ pressure from +10 mmHg above to −10 mmHg below eupnoea under control conditions and also during simulated orthostatic stress.

METHODS

Subjects

Ten healthy, non-smoking subjects (5 women, 5 men), aged 22 ± 3 (mean $\pm$ S.D.) years, participated in this study. The study requirements were explained in detail to all subjects, and all gave informed, written consent prior to participation. The experimental protocol was approved by the University of Wisconsin-Madison Health Sciences Human Subjects Committee. Participants did not have pulmonary, neurological, or cardiovascular disease, as assessed by history and physical examination.

General procedures

All experiments were conducted with subjects lying supine with their lower extremities, up to the level of the iliac crests, inside an airtight lower body negative pressure (LBNP) chamber. The room temperature was maintained at $24 \pm 1\,^\circ$C. Ventilation was measured through a mouthpiece connected to a pneumo-tachograph (Model 3700; Hans Rudolph, Kansas City, MO, USA). Expired air was sampled from the mouthpiece and the end-tidal CO_2 tension (P_{ET,CO_2}) was measured with an infrared gas analyser (Model CD3A; Ametek, Pittsburgh, PA, USA). Heart rate was measured from the electrocardiogram. Blood pressure was measured indirectly on a beat-by-beat basis by photoelectric plethysmography (Finapres 2300; Ohmeda, Louisville, CO, USA) as well as at one minute intervals by an automated arm cuff sphygmomanometer (Dinamap 1846SX/P; Critikon, Tampa, FL, USA).

Measurement of cerebral flow velocity

A 2 MHz pulsed Doppler ultrasound system (Neurovision 500 M; Multigon Industries, Younkers, NY, USA) was used to measure peak cerebral blood flow velocity (CFV) in the proximal (M1) segment of the middle cerebral artery. The middle cerebral artery was insonated through the right temporal window using search techniques that have been described previously (Otis & Ringelstein, 1996). After obtaining the best-quality signal, the probe was secured using a headband device to provide a fixed angle of insonation.

Cerebrovascular responses to hyper- and hypocapnia

Baseline data were collected during at least 5 min of eupnoeic breathing with self-selected frequency and tidal volume. After a stable baseline was established, CO_2 was added to the inspirate until a P_{ET,CO_2} of +10 mmHg above baseline was attained. After 5 min of this level of hypercapnia, the subject's tidal volume and frequency were noted, and then s/he was instructed to maintain that same level of tidal volume and frequency, using audio and visual feedback, while the inspired CO_2 was reduced in steps to achieve P_{ET,CO_2} values of +5, 0, −5 and −10 mmHg relative to baseline eupnoeic breathing. At least 5 min of steady-state data were collected at each level of P_{ET,CO_2}.

We calculated mean values for CFV (velocity–time integral, see Data analysis, below) during steady-state eupcapnia, hypercapnia and hypocapnia. Linear regression analyses were performed to determine the slopes of the relationships between P_{ET,CO_2} and CFV. Separate slopes were computed for hypercapnia and hypocapnia. The day-to-day reproducibility of this measure of cerebrovascular responsiveness to CO_2 was assessed in a separate group of nine subjects (6 males, 3 females; aged 34 ± 11 years (mean $\pm$ S.D.). The group mean values for CO_2 response slopes measured on day 1 vs. day 2 were virtually identical (2.87 ± 0.18 vs. 2.91 ± 0.18 cm s^{-1} mmHg^{-1} for hypercapnia and 1.29 ± 0.10 vs. 1.14 ± 0.14 cm s^{-1} mmHg^{-1} for hypocapnia, both $P > 0.05$). The coefficients of variation (standard deviation of the difference scores/grand mean $\times$ 100) for hypercapnia and hypocapnia were 12 and 20%, respectively.

Experimental protocols

Each subject underwent a familiarization trial of LBNP prior to data collection. Then, the cerebrovascular response of the subject to hyper- and hypocapnia was assessed twice: once with LBNP maintained at −40 mmHg and again without LBNP. The order in which the two trials were performed was randomized (in four subjects the LBNP trial was performed first, and in six subjects the no-LBNP trial was performed first). At least 5 min of baseline data were acquired before initiation of the CO_2 response tests. After the CO_2 response tests were completed, six of the subjects performed 20 s-breath holds starting from functional residual capacity in the control condition and during LBNP at −40 mmHg (3–4 trials in each condition). We previously determined the day-to-day reproducibility of CFV increases during 20 s-breath holds (coefficient of variation, 20%).

All variables were recorded continuously on paper (Astro-Med K2G; Grass Instruments, West Warwick, RI, USA) and videotape (Model 400A PCM; Vetter, Rebersburg, PA, USA). The signals were also routed to a computer (sampling rate, 120 Hz) for off-line analysis using custom-written software.

Data analysis

Measurements were performed using custom-made software. Mean CFV for each cardiac cycle was determined from the integral of the maximal frequency shift over one cardiac cycle divided by the length of the corresponding cardiac cycle (i.e. velocity–time integral). The beat-by-beat values for CFV, breath-by-breath values for P_{ET,CO_2} and minute-by-minute values for mean arterial pressure (MAP) obtained with the arm cuff sphygmomanometer, calculated as 1/3 pulse pressure + diastolic pressure, were averaged over the 5 min at each level of CO_2. The slopes of the linear relationships between CFV and P_{ET,CO_2} during hyper- and hypocapnia in the 0 and −40 mmHg conditions were compared by Student's paired t tests. Five minute averages of MAP were compared using a 2-way analysis of variance with repeated measures on the CO_2 and LBNP factors. To assess the CFV and MAP responses to breath holds, the peak values at the termination of each breath hold were computed as three-beat averages of the actual highest cardiac cycle and the two adjacent cardiac cycles. The change in CFV and MAP caused by each breath hold was computed as the difference between the peak value and the mean of the baseline values. For each subject, haemodynamic responses to the 3–4 breath hold trials in each condition were averaged and these average values were used in computation of the group means. P values < 0.05 were considered statistically significant. Except where otherwise noted, data are expressed as means $\pm$ S.E.M.

RESULTS

Haemodynamic and ventilatory responses to LBNP alone

Application of LBNP at −40 mmHg produced an increase in heart rate and a decrease in pulse pressure with no change in MAP (Table 1; $n = 10$). Application of LBNP produced an increase in minute ventilation that was caused mainly by an increase in tidal volume. This increase in minute ventilation resulted in a decrease in P_{ET,CO_2}. CFV
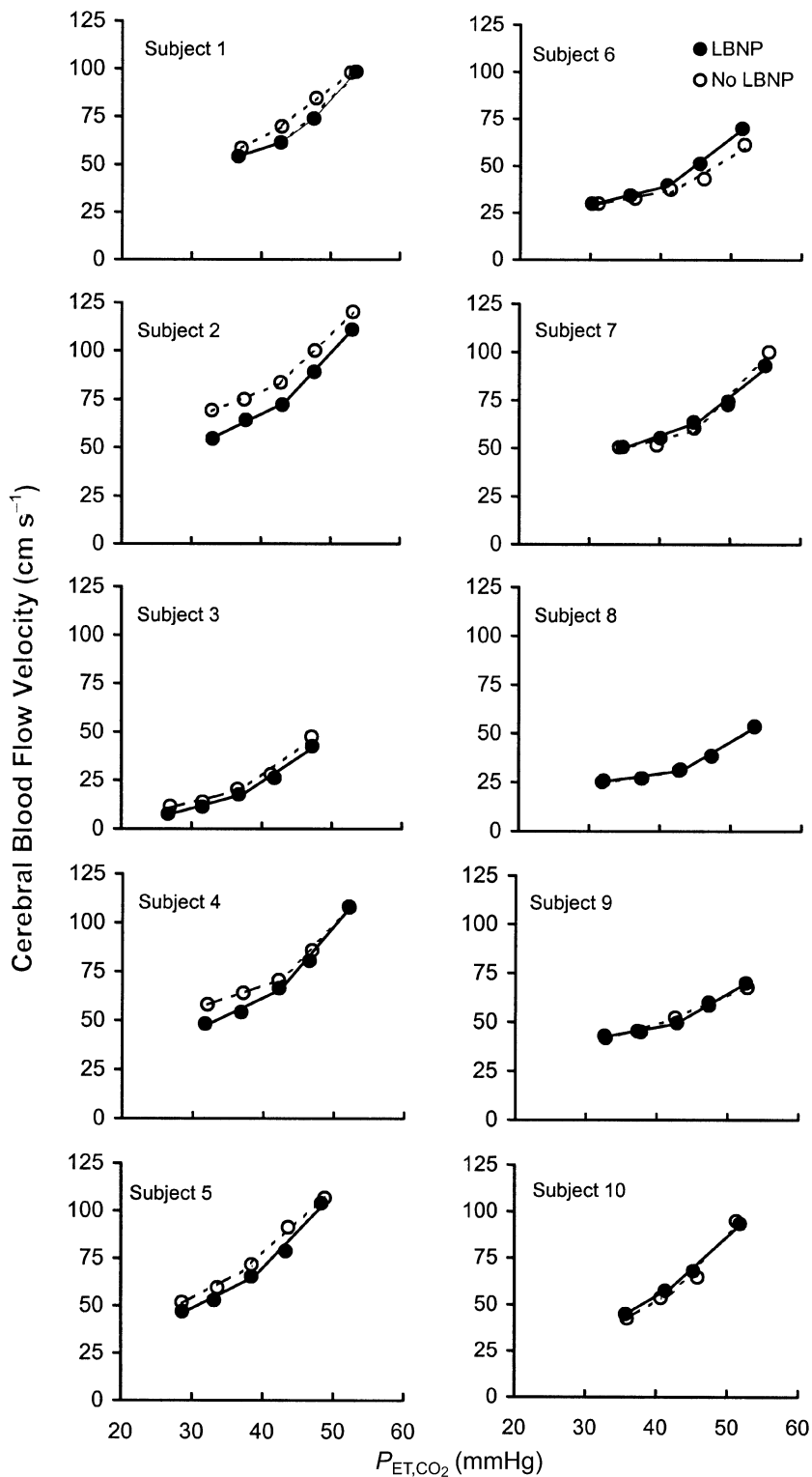
Figure 1. Individual cerebral flow velocity responses to hyper- and hypocapnia with (●) and without (○) lower body negative pressure (LBNP)

Continuous and dashed lines represent linear regressions of velocity on P_{ET,CO_2}, calculated separately for hyper- and hypocapnia.

Table 1. Haemodynamic and spontaneous ventilatory responses to LBNP at –40 mmHg

LBNP (mmHg)	HR (beats min^{-1})	MAP (mmHg)	PP (mmHg)	V_E (l min^{-1})	V_T (l)	R_f (breaths min^{-1})	P_{ET,CO_2} (mmHg)	CFV (cm s^{-1})
0	77 ± 8	81 ± 3	63 ± 6	7.6 ± 0.7	0.5 ± 0.1	15 ± 1	40.5 ± 1.1	53.5 ± 9.2
–40	$93 \pm 9^*$	85 ± 5	$54 \pm 5^*$	$11.5 \pm 1.5^*$	0.9 ± 0.2	16 ± 1	$31.9 \pm 1.7^*$	$40.7 \pm 6.9^*$

Note that during these measurements no attempt was made to control V_T, breathing frequency, or P_{ET,CO_2}. HR, heart rate; PP, pulse pressure; V_E, ventilation; V_T, tidal volume and R_f, breathing frequency. $n = 10$; $^*P < 0.05$, –40 *vs.* 0 mmHg LBNP.

Table 2. Haemodynamic and ventilatory responses to LBNP at –40 mmHg when ventilation was maintained at the level elicited during hypercapnia (P_{ET,CO_2} +10 mmHg above eupnoea) and P_{ET,CO_2} was held constant at control levels

LBNP (mmHg)	HR (beats min^{-1})	MAP (mmHg)	PP (mmHg)	V_E (l min^{-1})	V_T (l)	R_f (breaths min^{-1})	P_{ET,CO_2} (mmHg)	CFV (cm s^{-1})
0	70 ± 6	80 ± 2	61 ± 4	29.6 ± 5.6	1.5 ± 0.3	20 ± 1	41.6 ± 0.8	55.0 ± 6.4
–40	$88 \pm 6^*$	83 ± 2	$54 \pm 3^*$	29.8 ± 5.5	1.5 ± 0.3	20 ± 1	41.6 ± 0.8	52.4 ± 5.6

$n = 10$; $^*P < 0.05$, –40 *vs.* 0 mmHg LBNP.

was lower than baseline during LBNP at –40 mmHg when subjects breathed spontaneously (i.e. they hyperventilated); however, when P_{ET,CO_2} was returned to the control level via supplementation of the inspired air, CFV was the same as in the no-LBNP condition.

Responses to hyper- and hypocapnia without LBNP

In all subjects, supplementation of the inspired CO_2 produced graded increases in CFV, whereas voluntary hyperventilation caused graded reductions in CFV (Figs 1 and 2, open circles). Mean values for the slopes of the linear relationships between CFV and P_{ET,CO_2} were

Figure 2. Relationships between P_{ET,CO_2} and CFV and mean arterial pressure (MAP) with (●) and without (○) LBNP

Data shown are means ± S.E.M., $n = 10$.

2.96 ± 0.3 cm s^{-1} $mmHg^{-1}$ for hypercapnia and 1.32 ± 0.19 cm s^{-1} $mmHg^{-1}$ for hypocapnia.

The higher level of hypercapnia (P_{ET,CO_2}, +10 mmHg above baseline) produced small, but consistent elevations in MAP ($P < 0.05$). MAPs at all other levels of P_{ET,CO_2} were not different from baseline ($P > 0.05$; Fig. 2).

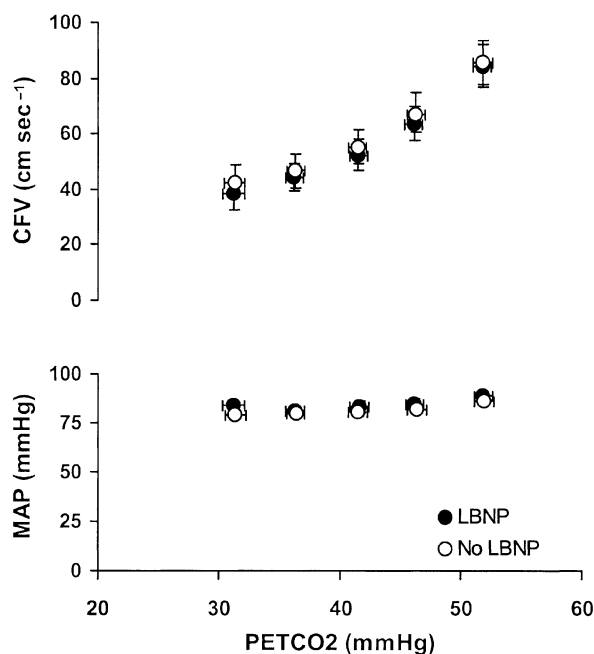### Responses to hyper- and hypocapnia during LBNP

Since our goal was to compare CFV responses over the same range of P_{CO_2} values with and without LBNP, we supplemented the inspired CO_2 as necessary to restore baseline P_{ET,CO_2} prior to the initiation of the hyper- and hypocapnia trials. Baseline CFV, measured under isocapnic conditions at equal tidal volumes and breathing frequencies, was unaffected by application of LBNP (55 ± 6 and 52 ± 6 cm s^{-1}, $P > 0.05$; Table 2).

Application of LBNP did not alter the slope of the CFV responses to hypercapnia (3.14 ± 0.24 *vs.* 2.96 ± 0.26 cm s^{-1} $mmHg^{-1}$, $P > 0.05$) or to hypocapnia (1.31 ± 0.18 *vs.* 1.32 ± 0.19 cm s^{-1} $mmHg^{-1}$, $P > 0.05$; Fig. 2). The order of trials had no effect on CFV responsiveness. The between-trial difference in slopes was the same in subjects who performed the LBNP trial first *vs.* those who performed the LBNP trial second, both for hypercapnia (0.03 ± 0.31 *vs.* 0.27 ± 0.18 cm s^{-1} $mmHg^{-1}$) and for hypocapnia (0.19 ± 0.08 *vs.* − 0.14 ± 0.17 cm s^{-1} $mmHg^{-1}$) (both $P > 0.05$).

Application of LBNP did not affect the influence of alterations in P_{ET,CO_2} on MAP (Fig. 2). Similar to the no-LBNP condition, MAP was higher during the hypercapnia at +10 mmHg than at other levels of P_{CO_2} ($P < 0.05$).

Responses to breath holds with and without LBNP

LBNP had no effect on the increases in CFV evoked by 20 s breath holds (Fig. 3; $n = 6$; +50 ± 18 *vs.* +51 ± 19 %, $P > 0.05$). Likewise, the MAP responses to breath hold

were unaffected by LBNP ($+6 \pm 2$ *vs.* $+8 \pm 2$ mmHg, $P > 0.05$).

DISCUSSION

Summary of findings

In the present study we found that baroreflex-mediated increases in sympathetic vasoconstrictor outflow did not alter the cerebrovascular responses to hyper- and hypocapnia. Moreover, such sympathetic activation had no effect on the increase in CFV produced by apnoea. These findings, taken together with recent evidence from our laboratory that removal of basal sympathetic tone also had no effect on these responses (Przybylowski *et al.* 2003), calls into question the concept that sympathetic activation constrains, and sympathetic ablation enhances, cerebrovascular CO_2 responsiveness (Jordan *et al.* 1998, 2000). The following discussion details the assumptions and evidence that underlie these conclusions.

Critique of methods

Doppler ultrasonography measures flow velocity rather than blood flow. Nevertheless, we believe that velocity is a reasonable estimate of flow in our experiments because: (1) the diameter of the middle cerebral artery varies by less than ± 4 % during changes in arterial pressure (Giller *et al.* 1993), CO_2 tension (Giller *et al.* 1993; Valdueza *et al.* 1997; Serrador *et al.* 2000) or gravitational stress (Serrador *et al.* 2000), and (2) velocity and flow through the middle cerebral artery are highly correlated (Bishop *et al.* 1986; Kirkham *et al.* 1986). Our estimates of cerebrovascular CO_2 reactivity (6 % mmHg^{-1} for hypercapnia and 3 % mmHg^{-1} for hypocapnia) are consistent with previous reports based on measurements of tissue nitrous oxide uptake (Kety & Schmidt, 1948), functional magnetic resonance imaging (Rostrup *et al.* 1994; Kastrup *et al.* 2001), positron emission tomography (Ramsay *et al.* 1993; Nishimura *et al.* 1999), and ^{133}Xe washout (Tominaga *et al.* 1976).

We consider it unlikely that our negative findings were secondary to Type II hypothesis testing errors. The differences in cerebrovascular CO_2 response slopes during LBNP *vs.* no-LBNP were small ($+0.18 \pm 0.16$ cm s^{-1} mmHg^{-1} for hypercapnia and -0.01 ± 0.12 cm s^{-1} mmHg^{-1} for hypocapnia) and the 95 % confidence intervals for these differences (-0.18 to -0.54 cm s^{-1} mmHg^{-1} for hypercapnia and -0.27 to 0.26 cm s^{-1} mmHg^{-1} for hypocapnia) are both narrowly centred around zero difference, indicating that the true value of this parameter is very close to the null (Hoenig & Heisey, 2001). Furthermore, the coefficients of variation for the difference in CO_2 responses slopes of individual subjects (17 and 28 % for hyper- and hypocapnia, respectively) were similar to those observed in tests of the day-to-day reproducibility of these measurements. The order in which subjects were exposed

to the LBNP *vs.* no-LBNP conditions did not affect the difference in slopes.

Baroreflex effects on the cerebral circulation

Our conclusions are predicated on the assumption that LBNP, a stimulus that is known to increase sympathetic vasoconstrictor outflow to forearm, calf and splanchnic vascular beds via baroreflex unloading (Johnson *et al.* 1974; Abboud *et al.* 1979; Jacobsen *et al.* 1993), also increases sympathetic outflow to the cerebral circulation. From a teleological perspective, it seems inappropriate that baroreflex-induced sympathetic vasoconstriction, a mechanism for maintaining cerebral perfusion and avoiding syncope, would occur in the brain. Moreover, such vasoconstriction presumably could not assist in defending blood pressure in the face of orthostatic stress because the cerebral circulation is located above the level of the heart. Nevertheless, the neural substrate for such a reflex pathway is known to exist. Baroreflex deactivation increases efferent activity in the cervical sympathetic trunk (Tafil-Klawe *et al.* 1989) and electrical stimulation of the cervical sympathetic trunk causes vasoconstriction in multiple regions of the brain (Meyer *et al.* 1967). Although previous investigators demonstrated that carotid sinus baroreceptor activation had no effect on cerebral blood flow (Rapela *et al.* 1967; Heistad & Marcus, 1976), haemorrhage-induced baroreceptor deactivation (reductions in MAP to 60 and 45 % of baseline) did cause mild
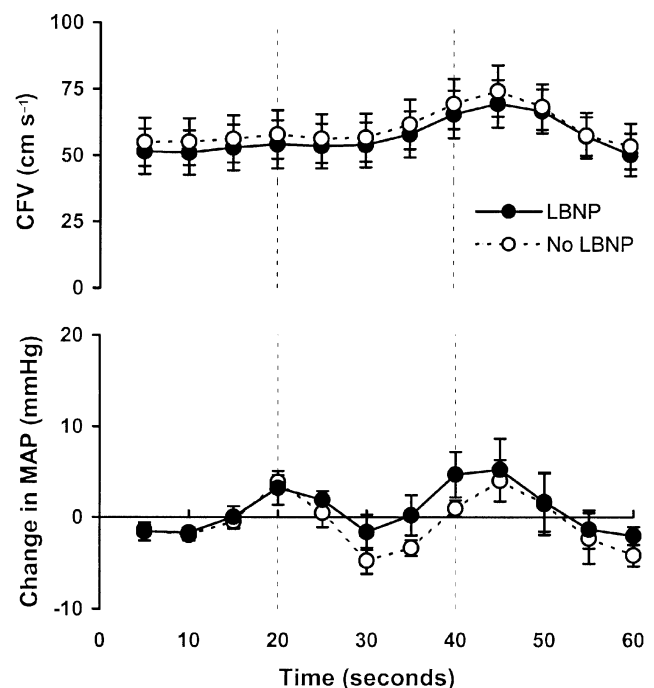
Figure 3. Cerebrovascular responses to 20 s breath holds with (●) and without (○) LBNP

The dashed vertical lines indicate the duration of the apnoea. Data shown are means ± S.E.M., $n = 6$.

cerebral vasoconstriction in anaesthetized cats (Gross *et al.* 1979). The smaller degree of baroreflex unloading produced by LBNP in our study (8 mmHg reduction in pulse pressure, no change in MAP), although equivalent to that produced by assumption of the upright posture, had no effect on baseline CFV or on the cerebrovascular responses to hyper- and hypocapnia.

It is important to note that other investigators have observed a decrease in CFV during immersion of the hand in ice water (cold pressor test) that was blocked by clonidine, an inhibitor of central sympathetic outflow (Micieli *et al.* 1994). In our study, activation of the sympathetic nervous system via LBNP-induced unloading of arterial and cardiopulmonary baroreceptors, alone and in combination with stimulation of chemoreceptors during breath hold, failed to alter cerebrovascular responsiveness to CO_2. We cannot, on the basis of our data, speculate whether other methods employed to increase sympathetic outflow in our study would be sufficient to influence CFV. In addition, because we measured cerebrovascular responses during sustained application of LBNP, it is possible that we may have overlooked the transient effects of abrupt increases in sympathetic outflow on CFV. In experimental animals, previous investigators have observed lack of sustained cerebral vasoconstriction (i.e. an 'escape' phenomenon) during electrical stimulation of the cervical sympathetic trunk (Baumbach & Heistad, 1983).

Effect of LBNP on cerebral perfusion pressure

Cerebral perfusion pressure, the difference between MAP and intracranial pressure when the latter exceeds central venous pressure (Weyland *et al.* 2000; Munis & Lozada, 2000), is a major determinant of CFV. Although we did not measure intracranial pressure or central venous pressure in our study, it is likely that both were reduced during LBNP (Jacobsen *et al.* 1993; Tankisi *et al.* 2002). If so, cerebral perfusion pressure would have been higher during LBNP *vs.* baseline conditions. The finding that CFV was unchanged in spite of increased perfusion pressure suggests that LBNP elicited an autoregulatory response. This autoregulation apparently had no affect on cerebrovascular CO_2 responsiveness, because the changes in CFV produced by alterations in P_{CO_2} were the same with and without LBNP.

Comparison with previous studies of cerebrovascular regulation during baroreceptor unloading

Our findings are consistent with those of previous investigators who observed comparable cerebrovascular CO_2 reactivity in the supine and sitting positions (Mayberg *et al.* 1996), but they conflict with those of a recent study that found reduced cerebrovascular CO_2 responsiveness during head-up tilt (Jordan *et al.* 2000). The reasons for this discrepancy are not obvious; however, there may be differences in the amount of sympathetic activation caused by actual *vs.* simulated orthostatic stress. In the previous study, sympathetic stimulation was produced by head-up tilt, a manoeuvre that not only unloads baroreceptors but also activates the vestibulosympathetic reflex (Ray & Monahan, 2002). In addition, in the previous study, CO_2 response slopes were calculated by linear regression of CFV on P_{a,CO_2} over the entire range of observed CO_2 tensions. It is possible that an apparent reduction in CO_2 responsiveness during head-up tilt occurred secondarily to the somewhat lower P_{CO_2} values observed during hyper- and hypocapnia during tilt *vs.* supine and the alinear nature of the cerebral blood flow:P_{CO_2} relationship (the response slopes are known to be steeper during hyper- than hypocapnia (Reivich, 1964)). In our experiments we ensured that P_{ET,CO_2} was the same in the sympathetic activation *vs.* no sympathetic activation conditions and we constructed separate slopes for responses to hyper- and hypocapnia.

Effects of sympathetic activation on baseline CFV: role of hypocapnia

Our finding that application of LBNP did not alter baseline CFV is also inconsistent with several other recent studies (Giller *et al.* 1992; Levine *et al.* 1994; Serrador *et al.* 2000). We suspect that this difference may be attributable to strict maintenance of eucapnia during LBNP in our subjects. In two of the previous studies, the P_{ET,CO_2} responses to LBNP were not reported (Giller *et al.* 1992; Levine *et al.* 1994). In the other study, LBNP-induced decreases in P_{ET,CO_2} occurred, but were not statistically significant (Serrador *et al.* 2000). In several other previous reports, hyperventilation-induced hypocapnia was a consistent, statistically significant finding during simulated orthostatic stress with LBNP and also during upright tilt (Kobayashi *et al.* 1980; Ahn *et al.* 1989; Cencetti *et al.* 1997; Cooke *et al.* 1999). In our subjects, we observed a drop in CFV at the onset of LBNP when P_{ET,CO_2} levels were allowed to fall; however, when we restored P_{ET,CO_2} to control by supplementing the inspired CO_2, we also restored the control level of CFV (Tables 1 and 2). The cause of hyperventilation during orthostatic stress, although not understood, may result from baroreflex–chemoreflex interactions (Somers *et al.* 1991) or perhaps the vestibulo-respiratory reflex (Monahan *et al.* 2002). Previous investigators speculated that this increase in ventilation reinforces the cardiovascular adjustments to central hypovolaemia by facilitating venous return via the respiratory pump and enhancing vasoconstriction via reduced P_{a,CO_2} (Hildebrandt *et al.* 2000).

In conclusion, we found that baroreflex-induced sympathetic activation had no influence on cerebrovascular responses to CO_2, one of the most powerful regulators of vascular tone in this region. Our findings do not exclude the possibility that sympathetic activation that is more intense or that is evoked via different mechanisms

might influence cerebral blood flow, although chemoreceptor activation during breath holds failed to uncover a difference. Nevertheless, they are consistent with the traditional view that the sympathetic nervous system plays a minor role, if any, in regulation of cerebral blood flow under physiological conditions. The major importance of sympathetically mediated vasoconstriction in the cerebral circulation may be to protect the blood–brain barrier when the limits of autoregulation are exceeded (e.g. during acute hypertensive episode; Mayhan *et al.* 1987).

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