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Variability in the Magnitude of the Cerebral Blood Flow Response and the Shape of the Cerebral Blood Flow: Pressure Autoregulation Curve during Hypotension in Normal Rats

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Background: **The maintenance of constant cerebral blood flow (CBF) as mean cerebral perfusion pressure (CPP) varies is commonly referred to as CBF-pressure autoregulation. The lower limit of autoregulation is the CPP at which the vasodilatory capacity is exhausted and flow falls with pressure. We evaluated variability in the magnitude of percent change in CBF during the hypotensive portion of the autoregulatory curve. We hypothesize that this variability, in normal animals, obeys a Gaussian distribution and characterizes a vasodilatory mechanism that is inherently different from that described by the lower limit.**

Methods: **Sixty-five male Sprague-Dawley rats were anesthetized with 0.5–1% halothane and 70% nitrous oxide in oxygen. Body temperature was maintained at 37°C. Using a closed, superfused cranial window, CBF (as % of control) was determined using laser Doppler flowmetry (LDF) through the window with the intracranial pressure set at 10 mmHg. Animals with low vascular reactivity to inhaled carbon dioxide and superfused adenosine diphosphate (ADP) or acetylcholine were excluded. MABP was sequentially lowered by exsanguination to 100, 85, 70, 55, and 40 mmHg. Using the %CBF *versus* CPP plots for each curve (1) the lower limit of autoregulation was identified; (2) the pattern of autoregulation was classified as "peak" (a rise in LDF flow of at least 15% as arterial pressure was dropped), "classic" (plateau with a fall), or "none" (a fall in LDF flow of greater than 15%); (3) the area under the autoregulatory curve between CPPs of 30 and 90 mmHg was calculated; and (4) the magnitude of the %CBF response to hypotension was assessed by determining the %CBF at a CPP of 60 mmHg ($\%CBF_{CPP60}$).**

Results: **Of the 65 curves, 21 had the peak pattern, 33 the classic pattern, and 11 the none pattern. The $\%CBF_{CPP60}$ and autoregulatory area displayed Gaussian distributions, consistent with normal variability. Although $\%CBF_{CPP60}$, autoregulatory area, and pattern were significantly correlated (r or $\rho > 0.84$, $P < 0.001$), the lower limit correlated weakly with autoregulatory area ($r = 0.34$, $P = 0.012$), and not at all with autoregulatory pattern or $\%CBF_{CPP60}$.**

Conclusions: **The $\%CBF_{CPP60}$ measures an aspect of the autoregulatory curve that is distinct from the lower limit. The peak autoregulatory pattern indicates that vessels are dilating more than is necessary to maintain a plateau in response to the pressure decrease, whereas the none pattern existed in spite of acceptable vascular responses to inhaled carbon dioxide and superfused ADP or ACh and the lack of surgical trauma. These results provide a different view of autoregulation during hypotension, are most likely dependent on the highly regional CBF method used, and could have implications concerning potential cerebral ischemia and hypotension during anesthesia.**

THE original concept of cerebral blood flow (CBF) pressure autoregulation (CBF autoregulation) was that CBF maintained a plateau, sometimes with a slight slope, between mean cerebral perfusion pressures (CPP) of 140 to 50 mmHg *via* a vasodilatory process, and then CBF becomes dependent on CPP, falling at pressures below the "lower limit".[1] Although this classic description is widely accepted,[2] variations in the lower and upper limits and the slope of the CBF *versus* CPP curve below the lower limit have been reported based on the analysis of individual, instead of mean, autoregulatory curves using a global CBF method.[3] In addition to these observations of variations in the lower limit of autoregulation, there are scattered observations of both paradoxical increases in CBF as blood pressure falls,[4–11] and of a linear relation between CBF and MABP,[8,12–15] all in physiologically normal subjects. Because these types of variations in the shape of the autoregulatory curve appear to be distinct from variations in the lower limit, we wish to define two terms with respect to the autoregulatory curve. The variations in CPP at the lower limit of autoregulation we describe as "horizontal variations" and the variations of CBF at the lower limit of autoregulation we describe as "vertical variations."

Previously we have shown that the lower limit is increased by cortical nitric oxide synthase inhibition,[16] similar to the results of Toyoda *et al.*[17] in the brain stem. During these observations, we noticed variations in the magnitude of the CBF response, or the vertical component of the autoregulatory curve, that agreed with the isolated observations of others as mentioned above. These vertical variations in CBF were more substantial than variations that occur in the lower limit, *i.e.*, horizontally, along the autoregulatory curve.

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We surmised that many observations of autoregulatory curves in the control setting would disclose variations from the classic pattern of autoregulation near the lower limit. We hypothesize that the lower limit does not provide an adequate measure of "vertical" autoregulatory function.

Methods

The methods are described in our previous publication,[16] but are repeated here with changes.

Animal Preparation and Cranial Window

Animal procedures were performed in conformance with the *Guide for the Care and Use of Laboratory Animals*[18] and were approved by the mandated institutional committee concerned with animal procedures. At the end of the experiment, animals were killed with a halothane overdose.

Installation of Cranial Window

To minimize the effects of possible trauma to the brain surface, a cranial window was implanted the day before experimental determinations. Sixty-five male Sprague-Dawley rats (380 ± 46 g, SD) were prepared the first day under 1 to 2% halothane and 70% N_2O anesthesia in oxygen, administered *via* endotracheal tube and intermittent positive pressure ventilation (Model 681, Harvard Apparatus, South Natick, MA). Thirty-seven animals were used previously in our investigation of the effect of nitric oxide synthase inhibition on the lower limit of autoregulation.[16] Rectal temperature was maintained at 37°C using a servocontrolled heat lamp (YSI 74, Yellow Springs, OH). After a midline scalp incision and removal of the periosteum, the surface of the skull was built up with dental acrylic and then flattened to provide a uniform surface for a methyl-methacrylate disk (1.5 mm × 10 mm with a 0.75-mm thick shelf on one side). An 8-mm diameter craniotomy was made with a high-speed drill, continuously cooled with a blast of nitrogen aimed carefully at the drilling site, and the dura mater removed. The 10-mm diameter cranial window, containing a glued-in thermocouple and three ports, two for artificial cerebrospinal fluid (aCSF) inflow and outflow and one for measuring epicortical pressure, was fixed to the skull with dental acrylic. The animal was given saline (10 ml kg^{-1} SC) to prevent postsurgical dehydration and prophylactic cefazolin sodium (1 mg kg^{-1} IM, Eli Lilly, Indianapolis, IN). The scalp was sutured, and the animal was allowed to recover from anesthesia. A suspension of the acetaminophen (McNeil, Fort Washington PA) in water (0.5 mg ml^{-1}) was presented as drinking water for the animal overnight.

Animal Preparation: Second Day

The next day the animal was anesthetized using 1 to 2% halothane and 70% N_2O in oxygen, a tracheotomy was performed, and artificial ventilation was instituted. Femoral arterial and venous catheters were inserted bilaterally. Halothane was reduced to a maintenance level of 0.5–0.9% to abolish the blood pressure response to tail pinch. Gallamine triethiodide (Davis-Geck, Wayne, NJ) in normal saline was infused intravenously at 10 mg kg^{-1} hr^{-1}. The animal was then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA).

Arterial blood gases ($Paco_2$, Pao_2, and pH) and hemoglobin concentration, (Hb), were determined with a blood gas analyzer (Model ABL3, Radiometer America, Westlake, OH). Arterial blood pressure was continuously monitored from a femoral artery, and epicortical pressure from the cranial window port, using strain gauge transducers (Model DT-XX, Viggo-spectramed, Oxnard, CA). The aCSF solution[19] was bubbled with 6% CO_2, 10% O_2, and 84% N_2 at 37°C, and pumped at 0.5 ml/min to the cranial window. It was reheated to 37°C just before entering the cranial window, as confirmed by monitoring the temperature in the cavity under the window with a thermocouple. The height of the outflow catheter was adjusted to provide a cranial window pressure of 10 mmHg. This intracranial pressure of 10 mmHg was subtracted from measurements of MABP to provide CPP. The Pco_2, pH, and Po_2 of the aCSF at the inflow catheter were 38 ± 4.1 mmHg, 7.389 ± 0.027, and 118 ± 22 mmHg, respectively. Epicortical temperature and pressure from under the cranial window, arterial blood pressure (both mean and pulsatile), end-tidal carbon dioxide, and CBF from laser Doppler flowmetry (LDF) were recorded on a polygraph (Model 2600S, Gould, Inc., Cleveland, OH) and on magnetic tape (VR-100–8EXP, Instrutech, Elmont, NY). MABP and CBF were sampled at one Hz by either a specially written computer program or by a data acquisition system (Codas, Dataq Instruments, Inc., Akron, OH) and stored on the computer hard drive. The tracings of MABP, end-tidal carbon dioxide, and CBF were displayed prominently for use in the vascular reactivity testing and hypotension protocol.

Laser Doppler Flowmetry

CBF was continuously monitored using Laser Doppler Flowmetry (LDF).[20] These measurements of CBF were obtained with a time constant of 1 s (Model BMP-403A, Vasamedics, St. Paul, MN; 780 nm wavelength, 1.6 mW output power) using a 0.8 mm diameter LDF probe (Model P-433–2). The probe was positioned just above the cranial window at a relatively avascular area using a micromanipulator attached to the stereotaxic frame. CBF values were expressed as a percentage of the control value (%CBF) after the LDF biologic zero, obtained after the termination of the experiment when the brain circulation was stopped, was subtracted.

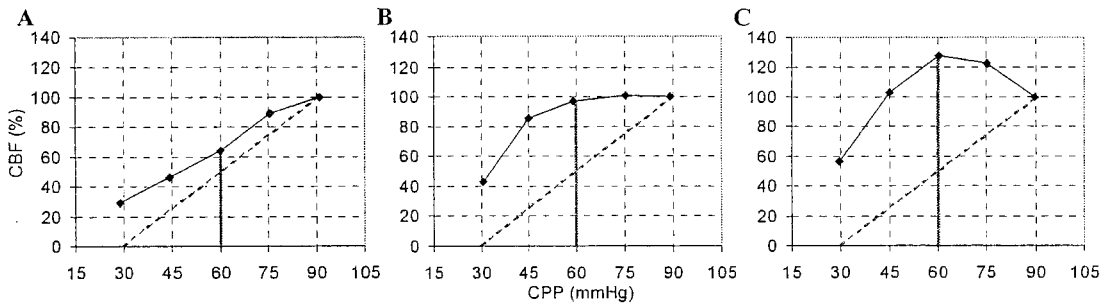

Fig. 1. The $\%CBF_{CPP60}$ area under the autoregulatory curve, and pattern are used to characterize the vasodilatory response to hypotension. This figure shows three typical autoregulatory curves, plotted as linear segments (solid line) connecting the five pairs of CPP and %CBF: panels A, B, and C show none, classic, and peak patterns, respectively. The line from zero %CBF at 30 mmHg CPP and 100 %CBF at 90 mmHg CPP (dashed line) was used as the baseline for the calculation of autoregulatory area. The solid vertical line shows the magnitude of the curve at 60 mmHg CPP, $\%CBF_{CPP60}$. The autoregulatory area and $\%CBF_{CPP60}$ are 982, 2301, 3500 mmHg-%CBF and 64, 97, 128 %CBF, respectively, for the none (*A*), classic (*B*), and peak (*C*) curves. The lower limits were 56 and 60 mmHg CPP for the classic (*B*) and peak (*C*) curves, respectively.

Vascular Reactivity Testing

Both the position of the probe and the integrity of the vasculature were tested by monitoring the CBF response to brief hypercapnia[21] during the initial control period. Inhaled carbon dioxide was increased to approximately 5%, just after an arterial blood gas sample was withdrawn ($Paco_2$pre). After end-tidal carbon dioxide indicated that a plateau had been reached, and if a brisk CBF response occurred, a second blood gas sample ($Paco_2$post) was drawn. If the carbon dioxide reactivity was absent, the probe was repositioned to another cortical area, and the carbon dioxide reactivity determined again. Once the carbon dioxide reactivity was acceptable, the response to either superfused 0.1 mM adenosine diphosphate (ADP) (in the first 40 animals) or superfused 0.01 mM acetylcholine (ACh) was tested (in the remaining 25 animals). Reactivities were calculated after the end of the experiment after correction for the biologic zero obtained at the termination of the experiment, using the equations: carbon dioxide reactivity = [100*(%CBFpost–%CBFpre)/(%CBFpre)]/($Paco_2$post - $Paco_2$pre) and ADP or ACh reactivity = 100*(%CBFpost - %CBFpre)/(%CBFpre). If carbon dioxide reactivity was less than 1% $mmHg^{-1}$, or if the ADP or ACh reactivity was less than 10% or 15%, respectively, the animal was excluded from the data set. Five animals were excluded because of low vascular reactivity and are not included in the data set.

Hemorrhagic Hypotension

Five levels of decreasing MABP were induced using step-wise hemorrhagic hypotension. After recording MABP, blood was withdrawn from the arterial femoral catheter into a heparinized syringe until a MABP of 100 mmHg was reached. This pressure was maintained for about 5 min by careful adjustment of the volume of blood in the syringe. This procedure was repeated for MABPs of 85, 70, 55, and 40 mmHg over a 25-min period. The mean total volume of withdrawn blood was 4.3 ± 1.5 ml. Ventilation was adjusted to maintain $Paco_2$ during controlled hemorrhage. Superfusion was continued with the aCSF during the entire sequence of hemorrhagic hypotension. Arterial blood gases were determined at the beginning (target MABP 100 mmHg) and end (target MABP 40 mmHg) of each blood-withdrawal.

Data Analysis

Determination of the pattern of autoregulation, the magnitude of the CBF response, and the area under the autoregulatory curve. For each blood-withdrawal sequence, five pairs of measurements of mean CPP and %CBF were taken over 128 sec periods of stable MABP obtained just before the next pressure drop. The CBF (in arbitrary units) at a CPP of 90 mmHg was used as the control (100%) %CBF value. A five point autoregulatory curve was constructed from these data points.

Three different methods were used to assess the vertical component of the autoregulatory curve. Two of these methods, autoregulatory pattern and area, require a full 5 point autoregulatory curve. The autoregulatory pattern was determined by an algorithm consisting of a series of expressions using inequalities and logical operators for each individual curve described by the five CPP *versus* %CBF data pairs. The "peak" pattern was assigned if there was at least a 15% increase in %CBF during the pressure drop, a "none" pattern if there was a continuous fall in %CBF of at least 5% in each successive data point with at least a 15% decrease by 60 mmHg, and a "classic" pattern for the rest. If an autoregulatory curve was classed as a "none" pattern, we attempted to find a sixth %CBF-CPP data pair at a higher pressure immediately previous to the first 90 mmHg CPP point. If a data pair from a higher pressure did exist, a six-point plot was produced, and the curve was then reassessed in an attempt to define a lower limit and pattern.

To calculate the autoregulatory area, the trapezoid rule was used to calculate the area between the autoregulatory curve and a linear line segment, as shown in figure 1. First, the area, A2, under a straight line from 30 mmHg

Table 1. Cortical Perfusate and Arterial Blood Variables by Autoregulatory Pattern

aCSF Perfusate	n	Pco_2, mmHg	Po_2, mmHg	*p*H
Mean of all groups	65	38 ± 4.1	118 ± 21.5	7.389 ± 0.027
Peak	21	39 ± 6.0	119 ± 19.1	7.379 ± 0.031
Classic	33	38 ± 2.7	118 ± 22.6	7.393 ± 0.023
None	11	38 ± 3.8	115 ± 24.2	7.399 ± 0.025

Animals	n	MABP, mmHg	$Paco_2$, mmHg	Pao_2, mmHg	*p*H	[Hb], g/dl
Control–mean	65	107 ± 7.3				
Peak	21	108 ± 7.1				
Classic	33	105 ± 6.7				
None	11	110 ± 8.3				
Pre-w/d–mean	65	99 ± 1.3	36 ± 2.0	126 ± 18.8	7.469 ± 0.034	18 ± 1.7
Peak	21	100 ± 0.7	37 ± 1.9	124 ± 20.0	7.462 ± 0.039	18 ± 1.1
Classic	33	99 ± 1.6	36 ± 2.0	125 ± 16.7	7.470 ± 0.032	18 ± 2.2
None	11	100 ± 1.0	35 ± 2.3	132 ± 22.4	7.476 ± 0.028	18 ± 1.1
Post-w/d–mean	65	40 ± 1.4	33 ± 3.3	138 ± 18.4	7.461 ± 0.042	15 ± 1.2
Peak	21	39 ± 1.4	33 ± 4.0	138 ± 19.4	7.466 ± 0.051	15 ± 1.3
Classic	33	40 ± 1.3	33 ± 2.9	137 ± 18.1	7.460 ± 0.038	16 ± 1.0
None	11	40 ± 1.8	33 ± 3.3	141 ± 18.7	7.454 ± 0.036	15 ± 1.3

For statistical analysis, see text.

aCSF = artificial cerebrospinal fluid; w/d = blood withdrawal.

CPP at a %CBF of zero to 90 mmHg CPP at a %CBF of 100 was determined and then subtracted from the area, A1, under each autoregulatory curve between CPPs of 30 mmHg and 90 mmHg, giving the area, A = A1 − A2 in units of mmHg-%CBF.

The third method, a more direct measure of the autoregulatory magnitude, required just two %CBF values at CPPs of 90 mmHg and 60 mmHg. For each autoregulatory curve, the %CBF at a CPP of 60 mmHg, $\%CBF_{CPP60}$, was used to characterize the magnitude of the CBF response to hypotension. Figure 1 presents typical none (fig. 1A), classic (fig. 1B), and peak (fig. 1C) autoregulatory curves with their $\%CBF_{CPP60}$, autoregulatory areas, and lower limits.

Data Analysis

Determination of the lower limit of autoregulation. For the determination of the lower limit of autoregulation, we used a procedure validated in our previous work,[16] where the intraclass correlation coefficient was 0.83. A separate autoregulatory curve for each animal was plotted without group or animal identification. Four blinded graders identified the lower limit of autoregulation, defined as the CPP just above the fall-off of %CBF. For a classic pattern, the lower limit was chosen as the pressure at which the plateau in %CBF starts to fall and for the peak pattern, the pressure at which %CBF starts to fall from the peak. If the curve was classed as a none, no lower limit was determined. The average of the lower limits from each grader was used for further analysis.

Chemicals

ADP and ACh were obtained from Sigma Chemical Co, St. Louis, MI.

Statistics

The Anderson-Darling test was used to assess normality of the distribution of $\%CBF_{CPP60}$ and autoregulatory area. The Spearman correlation coefficient (ρ) was used to assess the relation between the ordinal parameter, autoregulatory pattern, and the continuous variables $\%CBF_{CPP60}$, the autoregulatory area, and the lower limit. The Pearson correlation coefficient (r) was used to assess the relationship between continuous, normally distributed variables. Repeated measures analysis of variance was performed to test for differences between physiologic variables. Linear combinations of treatment means were used to test differences between groups. Paired or unpaired *t* tests were used for all comparisons. Values are expressed as mean ± SD. Statistical significance was assumed when *P* values were less than 0.05. The statistical analyses were performed using the SAS system (SAS Institute, Cary, NC).

Results

Physiologic Stability

There were no differences in physiologic variables between animals with peak, classic, and none patterns, as shown in table 1. Table 1 also presents the physiologic variables over time for each autoregulatory pattern. There were differences ($P < 0.001$) over time for MABP, Pao_2, [Hb], $Paco_2$, and pH. Because Pao_2 was maintained between 110 and 150 mmHg, oxygen saturation was always greater than 95%. The differences in MABP were intentional. Both $Paco_2$ and hemoglobin dropped as MABP was decreased from 100 mmHg to 40 mmHg. For instance, mean $Paco_2$ was 36 mmHg at 100 mmHg and 33 mmHg at the lower pressure. The mean hemoglobin

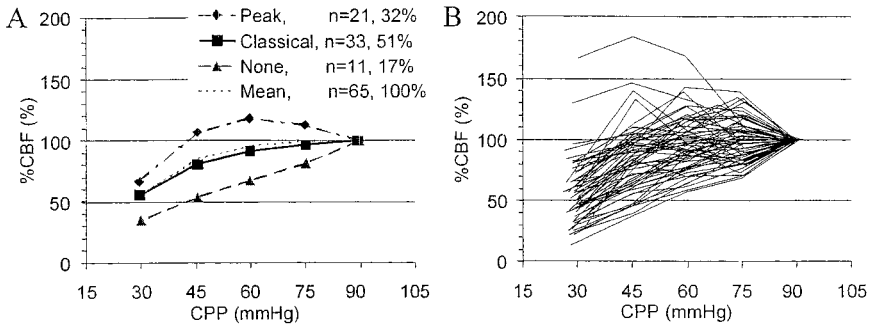

Fig. 2. Variations in the pattern of autoregulation are shown in plots of grouped (*A*) and individual (*B*) autoregulatory curves of CPP *versus* %CBF. These curves all start at a %CBF of 100% and a CPP of 90 mmHg. Panel A shows the mean curves of the three patterns, peak (n = 21), classic (n = 33), and none (n = 11), along with the mean curve (n = 65, mean), which is similar to the usual description of autoregulation. Panel B shows the autoregulatory curves of all animals plotted individually showing that these curves do not fall into three categories but are spread evenly between the extremes of the peak and none patterns.

difference during imposed hemorrhagic hypotension was 2.5 g dl^{-1}, and for pH, 0.009.

Patterns of Autoregulation

There were 12 autoregulatory curves originally classed as nones for which a sixth point %CBF-CPP data point at a pressure higher than 90 mmHg was sought. Such a high pressure point was found in six animals: one of these six curves was reclassified as a classic pattern using the 6-point plot.

The three patterns of autoregulation are demonstrated in figure 2A. The autoregulatory curves (n = 65) are plotted as divided into the patterns of peak (n = 21, 32%), classic (n = 33, 51%), and none (n = 11, 17%), along with the mean curve of all three groups. The mean lower limits for the classic and peak patterns were similar (60 ± 13 and 61 ± 11 mmHg CPP, respectively); thus, the mean lower limit in the 54 animals with peak and classic patterns (the none pattern does not have a lower limit) was 61 ± 12 mmHg CPP. These same curves are plotted individually in figure 2B. The plot of all of the individual curves in figure 2B shows a uniform distribution between the extremes of the peak to none patterns. The mean $\%CBF_{CPP60}$ for the peak, classic, and none patterns are 118 ± 17, 91 ± 10, and 68 ± 8 %CBF, respectively, and the mean $\%CBF_{CPP60}$ of the 65 curves is 96 ± 21%CBF. The frequency distributions of the $\%CBF_{CPP60}$ and the autoregulatory area are similar to the Gaussian distribution ($P > 0.25$, $P > 0.25$, Anderson-Darling test), as shown in figure 3.

The three methods of assessing the magnitude of the CBF response during hypotension, $\%CBF_{CPP60}$, the autoregulatory area, and the pattern, all compare well with each other, with Spearman or Pearson correlation coefficients of greater than 0.84 ($P < 0.001$), as shown in table 2. In contrast to the strong correlation between these measures of the magnitude of the CBF response, the lower limit, as a gauge of when CBF falls, shows nonsignificant correlations with $\%CBF_{CPP60}$ and autoregulatory pattern, and a weak correlation with autoregulatory area (r = 0.34, $P = 0.012$).

Discussion

We observed substantial intersubject variations in the pattern or shape of the circulatory response to hypotension from the classic pattern usually associated with CBF-pressure autoregulation. These variations satisfy the criteria for a Gaussian distribution, and are not caused by surgical trauma to the brain or the lack of, or differences in, vasoactive responses to inhaled carbon dioxide or superfused vasodilators, ADP or ACh. These variations include both hyperautoregulation, which we have defined as the peak pattern, in which it can be assumed that there is greater vasodilation than that required to maintain blood flow, and the lack of vasodilation, defined as the none pattern, where CBF varied with chang-

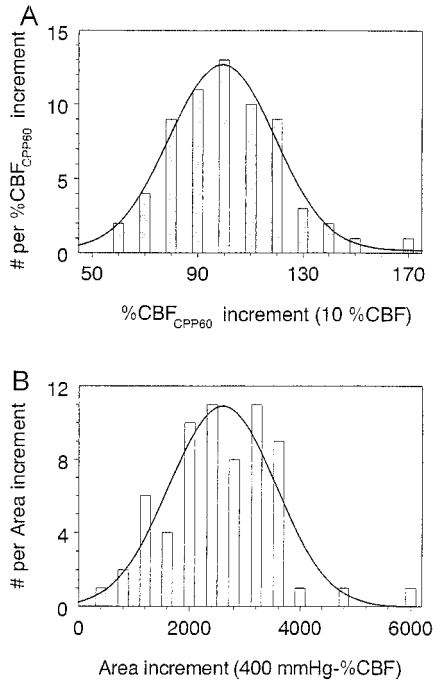

Fig. 3. The frequency histograms of the $\%CBF_{CPP60}$ (*A*) and the autoregulatory area (*B*) are compared with their normal distributions (solid lines) for the 65 normal autoregulatory curves. The similarity between the frequency histograms and the normal distributions suggests that the distribution of both the $\%CBF_{CPP60}$ and autoregulatory area are Gaussian distributions (Anderson-Darling test, $P > 0.25$, $P > 0.25$).

Table 2. Association between Methods of Assessing Autoregulation

Comparison	n	Pearson's Correlation Coefficient	Spearman's Correlation Coefficient	P Value
$\%CBF_{CPP60}$ *vs.* pattern	65		0.85	<0.001
$\%CBF_{CPP60}$ *vs.* autoregulatory area	65	0.92		<0.001
Autoregulatory area *vs.* pattern	65		0.84	<0.001
$\%CBF_{CPP60}$ *vs.* lower limit	54	−0.23		0.097
Autoregulatory area *vs.* lower limit	54	−0.34		0.012
Pattern *vs.* lower limit	54		−0.006	0.970

$\%CBF_{CPP60}$ = CBF at a mean cerebral perfusion pressure of 60 mmHg.

ing CPP in a pressure-dependent manner, as shown schematically in figure 4. When these individual autoregulatory curves are averaged together, the classic pattern results. Our data suggests that the autoregulatory pattern and $\%CBF_{CPP60}$ present additional descriptors of the normal physiologic phenomena of CBF-pressure autoregulation in comparison with the lower limit.

Both the peak and none patterns have been reported by several investigators[13,22] in normal animals. The peak pattern is characterized by hyperautoregulation or a paradoxical rise in CBF as CPP dropped. It appears that vasodilation overcompensates as pressure is lowered. There are many scattered observations of hyperautoregulation using many CBF methods with hemorrhagic hypotension, in dogs,[4,9] baboons,[5] rats,[7,10] newborn lambs,[6] and cats.[8] These observations by other workers support our hypothesis that hyperautoregulation is a normal physiologic response and are consistent with our data from 65 autoregulatory curves in which a peak pattern was observed in 32% of the animals. The none pattern, observed in 17% of the autoregulatory curves, is characterized by CBF varying linearly with CPP as arterial pressure drops, indicating that no active process of vasodilation is occurring. Previous observations of the none pattern exist[8,14] as individual curves reported in the control group of normal subjects.

The wide variability in CBF response to hypotension documented in this work may have been under-reported for several reasons. Often these patterns have been obscured by averaging with each other. Analysis of autoregulatory data that is based on global CBF,[3] group means, or grouped individual curves[23] may hide the peak and none patterns. This is shown in a plot of our data in which the peak, classic, and none patterns are averaged into a mean curve which displays the classic pattern (fig. 2A). Most observations of these patterns in the literature are based upon CBF techniques that measure flow in a relatively small region of tissue, such as the LDF method used in this study.

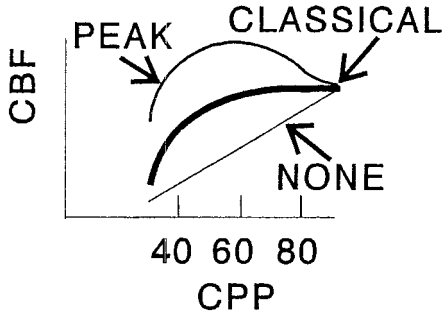

Fig. 4. A schematic representation three CBF *versus* CPP curves showing the relative frequency of the three autoregulatory patterns (peak, classic, or none) characterizing the vertical variations of autoregulation with the thickness of the lines indicating the frequency of observations. The classic pattern predominates, followed by the peak pattern, with the fewest none patterns.

Although in this work we contend that the none pattern is part of the normal spectrum of autoregulatory response, the none pattern, characterized as vasomotor paralysis with loss of autoregulation,[2] can occur as a result of brain damage. Thus the none pattern can be a result of severe trauma,[24] excessive increases in intracranial pressure,[15] experimental subarachnoid hemorrhage,[25] or lengthy focal cerebral ischemia,[26] increased intracranial pressure,[15,27] and the controlled cortical impact model of brain trauma.[22] Thus autoregulatory curves displaying linear relationships between CBF *versus* CPP have usually been excluded from analysis as "damaged preparations"[28] or as defective autoregulation[15] indicating a surgically traumatized brain or abnormally depressed vasodilatory mechanisms. As anecdotally reported by Rosenblum,[29] autoregulatory curves with the none pattern have been excluded from the literature, suggesting that the observance of the none pattern has been under-reported. However, in the animals in this study, the craniotomy and window placement were performed the previous day to avoid the disruption of cerebral circulatory regulatory mechanisms from surgical trauma, and intact and normal vasodilatory mechanisms to inhaled carbon dioxide and superfused ADP or ACh were documented. These normal vasodilatory responses would indicate that the cerebral circulation was physiologically normal, and suggest that the none pattern is part of a normal cerebrovascular response to hypotension. This suggestion is collaborated by the knowledge that the none pattern, when presented with the other patterns as $\%CBF_{CPP60}$ and autoregulatory area, exhibit a Gaussian distribution.

Most, if not all of the previous discussions of mechanism, including metabolic and myogenic, of autoregulation[30,31] have not dealt with the vertical aspect of auto-

regulation, but rather with the maintenance of the autoregulatory plateau between 60–150 mmHg CPP. Our previous work,[16] showing that the lower limit is increased by cortical nitric oxide synthase inhibition, suggests that nitric oxide is a "metabolic" autoregulatory vasodilator in this hypotensive range of blood pressure, so there is reason to suggest that nitric oxide plays a role in this vertical autoregulatory phenomena.

Because the original seminal description by Lassen[1] of CBF-pressure autoregulation of an autoregulatory plateau characterized by the maintenance of blood flow over a range of MABPs by active vasodilation and vasoconstriction, and the eventual precipitous drop of CBF below the lower limit, many workers have confirmed this basic pattern. This classic description of the cerebrovascular phenomena, or as slightly modified with a sloped plateau, is generally accepted as textbook knowledge both for research[2] and patient care.[32–34] In this study, we present observations that challenge this classic and accepted description.

The impact of variations in the value of the lower limit on clinical hemodynamic management has been discussed by Drummond.[35] However, given the relative constancy of the lower limit in comparison to the $\%CBF_{CPP60}$, autoregulatory area, or autoregulatory pattern, more concern for the effect of vertical variations in the autoregulatory pattern, rather than horizontal variations of the lower limit, might be warranted. Our data show that these vertical variations in $\%CBF_{CPP60}$ and autoregulatory area were more heterogeneous than variations in the lower limit: the coefficient of variation of the $\%CBF_{CPP60}$ (22%) and autoregulatory area (41%) are both greater than that of the lower limit (17%).

This work introduces an additional concept to characterize the CBF *versus* CPP curve that involves the height or magnitude of the CBF response. The $\%CBF_{CPP60}$, the autoregulatory area, and the pattern (peak, classic, and none) all relate to the magnitude of the CBF response to hypotension in slightly different ways, and differ from the commonly accepted and well-used lower limit. The heterogeneity displayed in figure 2B is characterized more effectively by these quantitative measures of the vertical variation in the CBF *versus* CPP curve than by the lower limit of autoregulation. This contention is supported by the fact that the lower limits of the peak and none pattern curves were both near 60 mmHg CPP and thus could not be used to distinguish between the patterns. Similarities between $\%CBF_{CPP60}$, the autoregulatory area, and the pattern, and differences of these from the lower limit are clearly demonstrated by the high Pearson's and Spearman's correlation coefficients of the parameters of CBF magnitude to each other (>0.84), compared to their lower coefficients with the lower limit, as shown in table 2. These similarities and differences in the ways of characterizing autoregulation imply that the magnitude of the response presents a different view of autoregulation.

Are the Patterns a Property of the Whole Brain or of Brain Regions?

Several studies lend support to the interpretation that the variations we have noted between animals are caused by variations within the cortex of each animal, and are not a property of the animal.[11,36] Does the fraction of large and small vessels, and arterioles and venules, near the LDF probe account for the different patterns of autoregulation? Although for our observations of the heterogeneity of autoregulation pattern, height, and area (figs. 2B, 3A, and 3B, respectively), the probe was positioned far from large vessels in a relatively avascular region of the cortex, the sensitive region measured by the LDF probe approaches the 1 to 2 mm spacing observed in oxygen heterogeneity in normal cat cortex by Wilson *et al.*[37]. This distance approaches the spacing between approximately two of Bär's medium vascular nodules of 300–700 μm.[38] The recent work by Lübbers *et al.*[39] shows oxygen heterogeneity of the same spacing. Thus, the distance of the LDF probe from vessels of different sizes could influence which autoregulatory pattern is observed. In this scenario, the heterogeneity of autoregulatory patterns and $\%CBF_{CPP60}$ is dependent on the heterogeneity of the surrounding vascular architecture.

Vascular Reactivity, Physiologic Variables, and Halothane

The reactivity limits for ADP and ACh were arbitrary; for carbon dioxide reactivity the value of 1% $mmHg^{-1}$ was chosen because it is considerably lower than the normal value of 2.5 to 3% $mmHg^{-1}$ [21]. The tests using carbon dioxide, ADP, and ACh reactivity were based on two separate vasodilatory mechanisms: carbon dioxide reactivity is exclusively mediated by nNOS[40,41] and ACh reactivity is exclusively mediated by eNOS in the endothelium.[42] We switched from ADP to ACh as our test for an endothelial dependent measure of vasoreactivity after we became aware that ADP stimulated dilation can operate without endothelium or with nitric oxide synthase inhibition.[43,44]

Both $Paco_2$ and hemoglobin were depressed as a result of hemorrhagic hypotension as shown in table 1. These changes in $Paco_2$ and hemoglobin occurred equally in the groups of autoregulatory pattern, so it is unlikely that they relate to these autoregulatory variations.

Although halothane has been shown to interfere with endothelium-dependent contraction in rat aortic rings,[45] there is little consensus on the effect of halothane on the phenomena of autoregulation. Whereas some studies report that the autoregulatory plateau is abolished with halothane,[46] several studies in rats using halothane concentrations between 0.4–1% show autoregulatory re-

sponses to be normal.[27,47,48] Surprisingly, Lee *et al.*[49] found that the autoregulatory plateau was flatter at the higher concentration of 1.5% halothane, than at 0.5%. Thus, in our study, the low halothane concentrations during the experimental phase speak against any influence of halothane on our results. In addition, minor variations in halothane concentration between animals of 0.5 to 0.9% were evenly distributed over the three groups of autoregulatory pattern and over the range of $\%CBF_{CPP60}$ values.

Implications for Cerebral Ischemia and Deliberate Hypotension

The differences and variations in the $\%CBF_{CPP60}$ and autoregulatory pattern observed in our data could have interesting and possible grave results. Since this is the first comprehensive description of vertical variations in the autoregulatory curve, the clinical implications are not completely clear. If our results are caused by regional differences in autoregulation responses, then as blood pressure falls downstream from arterial occlusion during thrombo-embolic stroke or during some other pathologic process such as trauma, or as a result of induced hypotension, the areas that exhibit the none response could be selectively vulnerable to ischemic pathology, whereas the areas that possess the peak response will be protected by the excess flow they receive at the lower pressures. If these different autoregulatory responses are a property of the entire brain rather than of individual regions of the brain, then the differences in autoregulatory capacity could explain pathogenic differences in between subjects. Consideration of these variations could partially explain selective vulnerability in the cortex if these variations are a regional phenomena that occur within the same subject.

These variations in the vertical component of the autoregulatory curve could affect the rationale and management of deliberate intraoperative hypotension. Deliberate hypotension has been used to minimize blood loss during surgical procedures when heavy blood loss is anticipated. The recommended minimum MABP is 50–55 mmHg,[50] which is based on the lower limit of autoregulation. However, our results suggest that this assumption could possibly be fine-tuned to the particular region or individual and that the shape of the curve should be considered in the choice of the minimum pressure in addition to the lower limit to avoid cerebral ischemia.

In conclusion, central features of the experimental design necessary to make these observations were (1) that each individual CBF response to hemorrhagic hypotension was analyzed without averaging, in contrast to analyzing only the mean response from a group of animals; and (2) that the none autoregulatory pattern with CBF linearly dependent on CPP is not necessarily indicative of a damaged, or nonphysiologic, cerebral circulation. Thus, these pressure-dependent (none) curves and over-vasodilated, hyperautoregulatory (peak) curves are part of a normal heterogeneous vasodilatory response to hypotension. The magnitude of the CBF response at 60 mmHg CPP, not the lower limit, characterizes these vertical variations in autoregulation. These different patterns of autoregulation may be important in understanding the mechanisms of the physiologic compensation that attempt to maintain sufficient CBF in the face of cerebral ischemia or induced or postural hypotension. These observations support a new and different interpretation of autoregulation and reveal either regional or between animal differences in the vertical component of the CBF *versus* CPP curve.

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