Relative Contributions of Sympathetic, Cholinergic, and Myogenic Mechanisms to Cerebral Autoregulation

J.W. Hamner, BS; Can Ozan Tan, PhD

Background and Purpose—Prior work aimed at improving our understanding of human cerebral autoregulation has explored individual physiological mechanisms of autoregulation in isolation, but none has attempted to consolidate the individual roles of these mechanisms into a comprehensive model of the overall cerebral pressure–flow relationship.

Methods—We retrospectively analyzed this relationship before and after pharmacological blockade of α-adrenergic–, muscarinic-, and calcium channel–mediated mechanisms in 43 healthy volunteers to determine the relative contributions of the sympathetic, cholinergic, and myogenic controllers to cerebral autoregulation. Projection pursuit regression was used to assess the effect of pharmacological blockade on the cerebral pressure–flow relationship. Subsequently, ANCOVA decomposition was used to determine the cumulative effect of these 3 mechanisms on cerebral autoregulation and whether they can fully explain it.

Results—Sympathetic, cholinergic, and myogenic mechanisms together accounted for 62% of the cerebral pressure–flow relationship (P<0.05), with significant and distinct contributions from each of the 3 effectors. ANCOVA decomposition demonstrated that myogenic effectors were the largest determinant of the cerebral pressure–flow relationship, but their effect was outside of the autoregulatory region where neurogenic control appeared prepotent.

Conclusions—Our results suggest that myogenic effects occur outside the active region of autoregulation, whereas neurogenic influences are largely responsible for cerebral blood flow control within it. However, our model of cerebral autoregulation left 38% of the cerebral pressure–flow relationship unexplained, suggesting that there are other physiological mechanisms that contribute to cerebral autoregulation. (Stroke. 2014;45:1771-1777.)

Key Words: autonomic pathways ▪ cerebrovascular circulation ▪ stroke
Although the studies used in this retrospective analysis were not explicitly designed with ANCOVA decomposition in mind, the minimal overlap in groups essentially fulfills the role of random assignment. Data from α-adrenergic, muscarinic, and calcium channel blockade were combined to elucidate the relative contributions of sympathetic, cholinergic, and myogenic mechanisms to the pressure–flow relationship. We then sought to determine whether the cumulative effect of these contributions is sufficient to explain autoregulation in healthy individuals fully.

Methods

A total of 43 volunteers (21–40 years old; 17 women) gave written informed consent before participation. There was no significant difference in subject characteristics (age, sex, and body mass index) between the 3 protocols (P>0.3 for all). Volunteers were healthy non-smokers free of cardiovascular or neurological diseases and not on prescription medications. All participants had abstained from caffeine for 12 hours and from alcohol and exercise for 24 hours before the study. All protocols were approved by the institutional review board and conformed to the Declaration of Helsinki. All studies were conducted between 8 AM and 12 PM.

Experimental Protocols

After inserting a 20-gauge catheter into an antecubital vein for drug infusion, volunteers were instrumented for electrocardiographic lead II (Dash 2000; General Electric), beat-by-beat photoplethysmographic arterial pressures (Finapres; Ohmeda), and oscillometric brachial pressures (DASH 2000; General Electric) as a calibration for the photoplethysmographic measures. A transcranial Doppler ultrasonograph (2 MHz probe; DWL MultiDop T2) was used to measure cerebral blood flow velocity at the M1 segment of the middle cerebral artery at a depth of 50 to 65 mm. Prior work suggests that the diameter of the middle cerebral artery remains relatively constant, despite changes in blood pressure induced by lower body negative pressure. Thus, flow velocity was used as a surrogate for flow. Expired CO2 was continuously monitored by an infrared CO2 analyzer (vacuumed) connected to a nasal cannula. All signals were digitized at 1 kHz (PowerLab, ADInstruments).

Data were collected during 5 minutes of supine rest followed by 10 minutes of oscillatory lower body negative pressure at a moderate level (−30 to −40 mm Hg) at 0.03 Hz (ie, ~30 seconds) oscillations. This level of oscillatory lower body negative pressure results in pressure oscillations comparable to those that occur within a physiological range and reliably engage cerebrovascular regulatory mechanisms. The same protocol was repeated after pharmacological blockade.

Pharmacological Blockades

Data were collected at baseline (n=48; twice in 5 subjects to account for within-subject variability) and after intravenous phentolamine (0.14 μg/kg bolus followed by 0.014 μg/kg per minute infusion; sympathetic blockade, n=11), glycopyrrolate (stepwise injections of 0.2 mg for 20–30 minutes to reach a stable mean heart rate >100 bpm; cholinergic blockade, n=9), and nicardipine (3 mg bolus infusion for 8–10 minutes; myogenic blockade, n=16). At these doses, phentolamine blocks α-adrenergic effects on the vasculature, glycopyrrolate blocks muscarinic receptors present on the endothelium of the pial arteries without central cholinergic impairment, and nicardipine blocks calcium channels on the vascular smooth muscle. Seven volunteers did not receive any infusions, and so, only their baseline data are included in subsequent analyses.

Data Analysis

Although linear methods are not sufficient to quantify the pressure–flow relationship when autoregulation is effective (because of the nonlinear nature of this relationship), the complexity of most nonlinear approaches precludes simple physiological interpretation of the observed relationships. To overcome these limitations, we used projection pursuit regression (PPR; online-only Data Supplement). Prior work has shown that PPR consistently reveals the dominant nonlinearity in the pressure–flow relationship. Subsequent parametrization allows identification of points where the relationship changes from seemingly more passive regions (ie, higher slopes) to more active regions (ie, slopes approaching zero) of autoregulation. The slope of the pressure–flow relationship within each region provides a measure of the effectiveness of autoregulation within that region (lower slopes indicate more effective counterregulation of pressure fluctuations). Therefore, PPR allowed us to define the characteristic nonlinear pressure–flow relationship via 5 markers (falling slope, rising slope, lower and upper pressure limits of the active region, and autoregulatory slope [or gain]; Figure 1) in a way that permits straightforward physiological interpretation of any alteration in this relationship under each condition (baseline versus different blockades).

We used ANCOVA decomposition to determine the relative contribution of sympathetic, cholinergic, and myogenic mechanisms to autoregulation. We asked whether there is a differential change in the characteristic nonlinear pressure–flow relationship after blockade of each mechanism. The relationship after each blockade would be reflective of the other 2 (unblocked) mechanisms (in addition to any other mechanism that is unaccounted for). This is analogous to a multiple regression wherein the pressure–flow relationships at baseline are predicted from data pooled from (1) cholinergic and myogenic blockades (reflective of sympathetic effect), (2) sympathetic and myogenic blockades (cholinergic effect), and (3) sympathetic and cholinergic blockades (myogenic effect). ANCOVA refines estimates of individual variations in the pressure–flow relationship at baseline and adjusts the treatment effects for any differences between the blockade groups that may have existed before the treatments. ANCOVA is routinely used with experimental designs, such as ours, wherein variables of interest...
Statistics
Data were analyzed using Matlab (version 7.10; Mathworks, Natick, MA) and in R-Language. Normal distribution was confirmed via Q–Q plots, and Box–Cox transformation was applied when necessary. For ease of interpretation, all values presented in the text and tables are to reduce cerebral blood flow (P<0.05; Figure 2), with significant contributions from each of the 3 effectors (partial $R^2$ of 0.20 for sympathetic, 0.11 for cholinergic, and 0.31 for myogenic effectors). ANCOVA decomposition demonstrated that (1) the lower limit of the active region of autoregulation can be attributed primarily to myogenic effectors, whereas its upper limit depends exclusively on neurogenic mechanisms; (2) effectiveness of autoregulation (ie, the gain) within the active region primarily depends on sympathetic mechanisms, with some contribution from myogenic and cholinergic effectors; and (3) the slope of the pressure–flow relationship outside this region is mostly determined by myogenic effectors.

Sympathetic blockade markedly linearized the autoregulatory response by increasing gain within the active region (from 0.02±0.05 [SE] to 0.55±0.09 cm s$^{-1}$ mm Hg$^{-1}$; P<0.05; Figures 3 and 4). Cholinergic blockade primarily resulted in an overall linearization of autoregulation resulting in an increased range (from 7.7±0.5 to 11.4±0.9 mm Hg; P<0.05) and gain (to 0.28±0.08 cm s$^{-1}$ mm Hg$^{-1}$; P<0.05) within the active region. Both sympathetic and cholinergic blockades tended to increase the slope of the relationship between pressure and flow above the active region (ie, increased the rising slope), although this effect reached significance only in case of cholinergic blockade. Myogenic blockade markedly reduced the pressure range of the active region (to 3.7±0.7 mm Hg; P<0.05) primarily by increasing the lower limit of this range and reduced the pressure–flow relationship above and below the active region (ie, reduced the rising and falling slopes). Both cholinergic and myogenic blockades also increased the gain within the active region of autoregulation but to a lesser extent compared with sympathetic blockade.

Results
A 1-way ANOVA by blockade (Table) showed a significant effect of blockade type on R-R interval (all blockades P<0.05) and an effect of only sympathetic blockade on expired CO$_2$. Sympathetic and cholinergic blockade had a tendency ($P=0.09$ and $P=0.10$, respectively) to cause a physiologically minor increase in blood pressure (3.2 mm Hg for both). Calcium channel blockade had a tendency to reduce cerebral blood flow (P=0.06) by ≈12%.

On an individual level, PPR fit the data well, explaining a majority of the relationship between pressure and cerebral blood flow ($R^2=0.60±0.02$; >0.50 in 70% of all data sets). Parameterization of each subject’s pressure–flow relationship provided the gain and range of the more active region of autoregulation, as well as the slope of the relationship outside of this region (Figure 1). There was no effect of blockades on the $R^2$ of the pressure–flow relationship (repeated measures ANOVA, $P=0.86$).

Overall, sympathetic, cholinergic, and myogenic mechanisms together accounted for 62% of the pressure–flow relationship ($P<0.05$; Figure 2), with significant contributions from each of the 3 effectors (partial $R^2$ of 0.20 for sympathetic, 0.11 for cholinergic, and 0.31 for myogenic effectors). ANCOVA decomposition demonstrated that (1) the lower limit of the active region of autoregulation can be attributed primarily to myogenic effectors, whereas its upper limit depends exclusively on neurogenic mechanisms; (2) effectiveness of autoregulation (ie, the gain) within the active region primarily depends on sympathetic mechanisms, with some contribution from myogenic and cholinergic effectors; and (3) the slope of the pressure–flow relationship outside this region is mostly determined by myogenic effectors.

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Discussion
This study took a comprehensive look at multiple physiological mechanisms responsible for cerebral autoregulation in humans. Our results suggest that although vascular myogenic control may be the largest determinant of the cerebral pressure–flow relationship, those effects occur outside the more active region of autoregulation, whereas neurogenic influences are largely responsible for cerebral blood flow control within it. Thus, although several studies have looked at whether or not individual pharmacological blockades abolish cerebral autoregulation, this work directly addresses how the sympathetic, cholinergic, and myogenic systems work in concert to shape the pressure–flow relationship in healthy humans.

Consistent with conventional wisdom, myogenic responses were the largest contributor to the pressure–flow relationship. Myogenic effects appeared to be concentrated in the slope of the cerebrovascular response to both rising and falling pressures, as well as being the primary determinant of the lower pressure limit of the active region of autoregulation. The sympathetic system

<table>
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<th>Blockade</th>
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<th>Myogenic (n=16)</th>
<th>Cholinergic (n=9)</th>
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<td>7/4</td>
<td>9/7</td>
<td>5/4</td>
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<td>R-R interval, ms</td>
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<td>828±36*</td>
<td>935±30*</td>
<td>643±39*</td>
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<td>Mean arterial pressure, mm Hg</td>
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<td>89.5±2.5</td>
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<td>89.5±2.6</td>
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<td>Cerebral blood flow, cm s$^{-1}$</td>
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<td>70.1±0.2</td>
<td>54±4.1</td>
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<tr>
<td>End-tidal CO$_2$, mm Hg</td>
<td>35.3±0.9</td>
<td>31.7±1.5*</td>
<td>34.6±1.3</td>
<td>35.9±1.3</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline.
represented the second largest contributor, via its effect on the gain and the upper pressure limit of the active region of autoregulation. The cholinergic system contributed in a relatively small but significant fashion toward the upper end of the active region (ie, gain and upper limit, and the rising slope). Hence, autonomic control of the cerebral vasculature is concentrated within the active region of autoregulation, with sympathetic and cholinergic reflexes accounting for 86% its gain and 99% of its upper limit. These results are broadly compatible with our previously published transfer function analysis that showed large effects of sympathetic blockade\(^6\) and smaller but qualitatively similar effects of cholinergic blockade.\(^7\) Current results provide further evidence for active autonomic control of the cerebral vasculature and suggest that it may be the most important factor in homoeostatic control of brain blood flow. This role for neurogenic mechanisms fits with their known effects; relatively constant blood flow in response to changes in pressure requires active changes in artery diameter, and sympathetic and cholinergic mechanisms are responsible for vasoconstriction and vasodilation. Thus, their involvement in determining the range and gain within the active region of autoregulation is easy to reconcile with our current understanding of their distinct but complementary effects.

Our recent work has shown that calcium channel blockade also has an effect on the pressure–flow relationship.\(^8\) The current study extends this finding and reveals its contribution to this relationship relative to that of neurogenic mechanisms. The myogenic response seems to be the main determinant of the lower limit of the active region of autoregulation and of the slope of the pressure–flow relationship outside this region. This is consistent with the concept of vascular myogenic pathways primarily responding to rapid changes in perfusion pressure and flow.\(^26\) Nonetheless, although the effect of myogenic mechanisms was largely outside of the active region, these seemingly passive regions are likely the most important regions in responding to changes in perfusion pressure that would otherwise lead to ischemia or hemorrhage. Furthermore, the myogenic role in these regions suggests that the tails of the pressure–flow relationship are not as passive as generally conceived.

It should be noted that 38% of the pressure–flow relationship at baseline remained unexplained after accounting for

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**Figure 2.** Contributions of neurogenic and myogenic mechanisms to cerebral pressure–flow relationship. Vertical bar shows their cumulative contributions (%) to overall relationship. Horizontal bars show the relative contribution of each mechanisms to different parameters of autoregulation.
neurogenic and myogenic effectors, suggesting that there may be other physiological mechanisms that contribute to cerebral autoregulation. One obvious possibility is that mechanical properties of vasculature can explain the remaining nonlinearity in the characteristic pressure–flow relationship. However, data from animals show that denervated middle cerebral artery devoid of myogenic tone responds passively to increasing pressure, although the amount of smooth muscle activation increases somewhat at higher pressures. These data suggest that in the absence of physiological effectors, blood flow should linearly track pressure, perhaps saturating at higher pressures. To verify this is also the case in human arteries, we explored the relationship between arterial pressure and brachial artery fluctuations after α-adrenergic blockade in 6 volunteers during an identical protocol. The responses of skeletal muscle resistance arteries to changes in arterial pressure are primarily under sympathetic control, and thus, the pressure–flow relationship after α-adrenergic blockade would mostly reflect intrinsic vessel properties. The brachial pressure–flow relationship after blockade was mostly linear (although some threshold and saturation effects were apparent; Figure 5). Therefore, intrinsic resistance artery properties cannot explain the remaining nonlinearity in the characteristic pressure–flow relationship after neurogenic and myogenic blockades.

Another likely candidate is endothelial nitric oxide (NO). However, studies exploring the possible role of NO in cerebrovascular control have provided inconclusive findings. For example, intravenous infusion of the NO donor sodium nitroprusside does not affect cerebral autoregulation in response to pressure changes, and blockade of NO-mediated pathways has not clarified the importance of this possible regulatory mechanism. Although NO synthase blockade does not change the relationship between spontaneous pressure and flow fluctuations, it does blunt the cerebral blood flow responses to ischemic thigh cuff release. However it is possible that, much like calcium channel blockade, changes in the pressure–flow relationship after NG-monomethyl-L-arginine (L-NMMA) are not reflected in linear approaches to cerebral autoregulation (eg, transfer function gain). Thus, the role of NO in cerebral autoregulation in humans remains uncertain, and further investigation is warranted.

A third potential factor that might account for the remaining pressure–flow relationship after neurogenic and myogenic blockade is multiplicative, or second-order, interactions between these effectors. Our assumption that the relationship after each blockade would be reflective of the other 2 (unblocked) effectors is a reasonable one, but we cannot account for the fact that blockade might augment (or dampen) the unblocked systems. For example, the cholinergic system might play a permissive
role to nitric flow regulation rather than acting as a direct mediator of the vasomotor response. Nevertheless, although the exact nature of interactions between the physiological systems we examined cannot be determined without multiple simultaneous (and possibly dangerous) pharmacological blockades, our work does establish their individual importance in cerebral autoregulation. Future work should attempt to tease apart possible interactions between the mechanisms of cerebral autoregulation.

A final consideration is that while we used pharmacological doses reported in the literature to effect complete blockade, it is nevertheless possible that we did not achieve it in every subject. However, if this is the case, our results suggest that we may have underestimated the ability of the sympathetic, myogenic, and cholinergic systems to explain cerebral autoregulation. This possibility should be noted in case future work is unable to establish an important role for NO or other mechanisms.

Limitations

Although PPR explained a majority of the pressure–flow relationship at the individual level, it did not account for the variation in this relationship fully. Nevertheless, the purpose of our approach is not to predict cerebral blood flow to its fullest extent but rather to characterize the nature of the pressure–flow relationship accurately in a physiologically meaningful way. Another consideration is that the cerebral pressure–flow relationship is frequency dependent. We examined the relationship between fluctuations only at 0.03 Hz because of technical limitations on how slowly oscillatory lower body negative pressure can cause pressure to fluctuate and because autoregulation is active at this frequency. Nonetheless, it is possible that the relative contribution of these physiological systems may be frequency dependent as well. Although frequencies faster than 0.07 Hz are of little interest because they are not counterregulated against, oscillations slower than 0.03 Hz could be important to examine. An additional limitation to this study is that while 48 data sets were analyzed, the number of subjects in each blockade group ranged from 9 to 16. It is possible that with more subjects the marginal $P$ values ($P<0.10$) of some of the minor hemodynamic effects of blockade would have achieved significance, but such a change would not alter the interpretation of our main findings.

**Figure 4.** Average ($±SE$) change in parameters of cerebral autoregulation at baseline and after each blockade. *$P<0.05$ vs baseline.
findings. Also note that although we use the term neurogenic when referring to the effect of blockade of $\alpha$-adrenergic and $\alpha$-adrenergic mechanisms, this term may be responsible for homoeostatic maintenance of cerebral blood flow. However, our model of cerebral autoregulation left 38% of the cerebral pressure–flow relationship unexplained, suggesting a role for other mechanisms, such as endothelial NO release, in cerebral autoregulation.

Conclusions

Our findings suggest that neurogenic control of the cerebrovasculature is largely responsible for responding to pressure changes that occur within the active region of autoregulation, whereas the effects of vascular myogenic control lie mostly outside this region. This suggests that neurogenic control may be responsible for homeostatic maintenance of cerebral blood flow, whereas myogenic control may be neuroprotective against ischemia and hemorrhage from rapid swings in pressure. However, our model of cerebral autoregulation left 38% of the cerebral pressure–flow relationship unexplained, suggesting a role for other mechanisms, such as endothelial NO release, in cerebral autoregulation.

Sources of Funding

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Disclosures

None.

References

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SUPPLEMENTAL MATERIAL

Data Analysis

Preprocessing

Cerebral blood flow and arterial pressure were recorded at 1 kHz and stored for offline data analysis. For data analysis, data were decimated to 5 Hz to accurately preserve the energy in the signal below 2.5 Hz. Although the dominant arterial pressure fluctuation occurs at the oscillatory frequency of lower body negative pressure (around 0.03 Hz in our case), random noise in the signals may interfere with the derivation of cerebral pressure – flow relationships (derivation of this relationship is described below). To increase the signal-to-noise ratio for the subsequent projection pursuit regression analysis, data were band-pass filtered at the oscillatory lower body negative pressure frequency (OLBNP) within a ± 0.005 Hz frequency band (see Supplemental Figure 1). For filtering, we used a first-order Chebyshev Type I with 1 dB of pass band ripple) around the frequency of OLBNP (0.03 Hz).

Projection Pursuit Regression Estimation

The cerebral pressure – flow relationship for each subject was derived by applying projection pursuit regression (PPR) to preprocessed data (using arterial pressure as the independent variable and middle cerebral artery blood flow velocity as the dependent variable). PPR has become a common technique for exploratory data analysis since its underlying mathematical principles and implementation were described and validated in the 1970s.¹ ²
PPR is a simple nonparametric technique that modifies a usual linear transfer function between the input \((x_t - \text{arterial pressure})\) and the output \((y_t - \text{cerebral blood flow velocity})\) (see equation 1 below). For each input \((x_t)\) and output \((y_t)\) a linear autoregressive transfer function (the term within the parentheses in equation 1) is passed through nonparametric kernel functions \(k_m\); called 'ridge functions'). Each kernel function \(k_m\) is a smoothing spline function of the same size as the data and determined by minimizing the mean squared error. Thus, PPR is an established atheoretical kernel method; meaning that a model is not posited \textit{a priori}, but derived directly from the observations (in this case, from arterial pressure and cerebral blood flow velocity measurements). This is a decided advantage for capturing a system whose physiology is not yet defined by explicit parametric models.

\begin{equation}
  y_t = f(x_t) = \sum_{m=1}^{M} \alpha_m k_m \left(\sum_1 \gamma^m_{i} x_{t-i}\right) \tag{1}
\end{equation}

Projection pursuit regression can include more than one ridge function (i.e., \(M > 1\) in equation 1). However, while more than one function will reduce the mean squared error, it may obscure the interpretation of ridge functions due to potential interactions between them. Because our primary purpose is to obtain a relation between arterial pressure and cerebral blood flow that can be interpreted physiologically (rather than predicting cerebral blood flow responses to its fullest extent), we limited PPR analysis to only one ridge function \((M = 1)\) (see Figure 1 in the main text).
Supplemental Figure II shows the resultant projection pursuit regression of one subject from data acquired during six different frequencies of OLBNP. Note that the relationship moves from essentially linear to resembling a “classic” Lassen curve as frequency decreases. It is possible that the range of autoregulatory region of the pressure – flow relationship further approaches the “textbook” curve as the pressure fluctuations become slower than 0.03 Hz. However, it is not feasible to generate controlled pressure oscillations below 0.03 Hz with OLBNP, as the cardiovascular system counter-regulates against the LBNP-induced arterial pressure changes before the cycle is finished.

**Piecewise Linear Parameterization**

The ridge function obtained for each subject is parametrized as a piecewise linear function for subsequent statistical analysis. This is achieved by fitting a Free-Knot B-spline to the ridge function via least-squares. B-splines, or basis-splines, are a piecewise polynomial functions of degree $k$ in a variable $x$ (in our case, the ridge function). The points where piecewise polynomial functions change are known as knots or break-points, and the number of knots is the minimum for the degree of the B-spline. Every function can be uniquely represented as a linear combination of B-splines of a given degree and smoothness. Moreover, when there is no theoretical basis for choosing a fitting function to describe a curve (in our case, the ridge function), the curve can be fitted with a spline function composed of a sum of B-splines, using the method of least squares. Given these considerations, and our aim to obtain a relation between arterial pressure and cerebral blood flow that can be interpreted physiologically, we
used first-order (that is, linear) piecewise functions as basis-functions to parametrize the ridge functions obtained from PPR. Note that determining the number of knots to use and where they should be placed is a constrained least squares problem which is linear in the spline coefficients but nonlinear in the free knots, and can easily be solved by the Schwetlick – Schütze algorithm.

Thus this step identifies on the ridge function those points where the arterial pressure - cerebral flow relationship changes, and the ranges wherein the relationship is approximately linear (see Figure 1 in the main text). The gain (i.e., the linear slope) of the pressure–flow relation within each region provides a measure of the effectiveness of cerebral autoregulation within that region. A lower gain indicates more effective counter-regulation of pressure fluctuations whereas higher gains indicate more passive flow responses to pressure changes.

**Reproducibility of Cerebral Pressure – Flow Relationship**

We have assessed the reproducibility of the cerebral pressure – flow relationship in a previous study. Five volunteers were asked to participate in a second study session, identical to the first (without drug administration). Both sessions were separated by a few weeks. After data collection and processing (as described above), we tested whether the nonlinear pressure – flow relation was consistent across the two separate study sessions. In particular, we assessed whether the effectiveness of autoregulation is reproducible across the two sessions. To that end, we used Lin’s concordance coefficient, \( \rho_c \), to test the null hypothesis \( (H_0) \) that the effectiveness of the autoregulation estimated across 2 separate study days are not in agreement (i.e., \( \rho_c < 0.9 \)). Lin’s concordance coefficient ranges from 0 (no concordance) to 1 (maximum concordance). It is
based on Pearson’s correlation coefficient (a measure of variation) but includes a bias correction term for systematic deviations. Thus, it is a stronger statistical test than least squares linear regression since it takes both the intercept and the slope into account. This analysis showed that the effectiveness of cerebral autoregulation i.e., the slope of the autoregulatory range) during 0.03 Hz OLBNP did not change ($\rho_c = 0.96, p < 0.001$ see Supplemental Figure III, and that the nonlinear pressure – flow relation is consistent across separate study days. Thus the characteristic relation between pressure and cerebral flow fluctuations assessed by this method were consistent across separate measurements.

**ANCOVA Decomposition**

In studies that involve differential expression of a trait associated with different pathways a global statistical test can avoid the problems of multiple testing while also accounting for possible correlations between observations. ANCOVA is one such test, wherein one can explore how observed parameters are influenced by design aspects of the study, and the observations may be correlated. The general framework involves parameters measured in $n$ samples with different covariates of interest. In our specific case, we had five parameters of the cerebral pressure – flow relation in 43 individuals (before and after pharmacologic blockade) and three covariates (sympathetic, myogenic, and cholinergic mechanisms).

From a purely statistical point of view, ANCOVA is just a replacement for the general multivariate linear model analysis in high dimensions. Its strength lies in its flexibility and ability to allow the inclusion of covariates that may correct for unbalanced situations. In particular, the
treatment groups do not have to come from the same population. The test is carried out by comparison of linear models via the extra sum of squares principle. That is, two statistical models are compared: the full model, which contains all covariates (baseline) and the reduced models, which do not have one covariate of interest (pharmacologic blockade).

The residual sum of squares (RSS) of the full model quantifies the ability of a model to explain the observed data. An orthogonal composition of the total sum of squares into treatment effects and error offers appropriate statistical tests for the null hypothesis that both full model and a given treatment effect explain the data equally well. If the explained variance of the full model is significantly greater than the reduced model then that covariate is deemed to make a significant contribution to the overall model. The relation of the sum of squares for each pharmacologic blockade to the RSS of baseline provides a measure of the contribution of each mechanism to the full model (see the left panel of Figure 2 in the main text). Subsequently, an orthogonal decomposition for the sum of squares for a given parameter provides relative contributions of each physiologic mechanism to the change observed in that parameter (see the right panel of Figure 2 in the main text).

For more details about the mathematical background and implementation of ANCOVA decomposition, we refer to the papers describing the approach in more detail.\textsuperscript{10} Adjustments for correlation between observations and multiple testing is implemented based on the method of Holm.\textsuperscript{11}
References


Supplementary Figure I: Data from a representative subject during 0.03 Hz OLBNP before and after preprocessing for PPR.
**Supplementary Figure II:** Data from a representative subject demonstrating the frequency dependence of the cerebral pressure–flow relation from 0.03 Hz to 0.08 Hz oscillatory LBNP.

<table>
<thead>
<tr>
<th>Change in blood flow (cm s(^{-1}))</th>
<th>90 - 110 mmHg</th>
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<tr>
<td>0.03 Hz</td>
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Supplementary Figure III: Autoregulatory gain from piecewise linear parameterization of PPR ridge functions from five subjects on two different study days.