Regional Cerebral Blood Flow During Hypercapnia in the Anesthetized Rabbit

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SUMMARY These experiments were designed to test the hypothesis that increases in blood flow to the lower brainstem would be greater than forebrain regions during arterial hypercapnia. Total and regional cerebral blood flow (CBF) was measured via the tracer microsphere technique in seven anesthetized New Zealand white rabbits during normocapnia (arterial PCO₂ = 40 torr) and hypercapnia (arterial PCO₂ = 80 torr). During normocapnia average CBF was 0.77 ml/min/g, and regional measurements of blood flow indicated significantly greater flow to the cerebrum (0.86 ml/min/g) than other areas of the brain. When arterial PCO₂ was increased average CBF increased 113%, and a significant linear regression was calculated for arterial PCO₂ vs CBF (CBF (ml/min/g) = 0.028 PCO₂ (torr) – 0.502). The distribution of blood flow within the brain was similar to normocapnia except that blood flow to the cerebellum was now greatest than in any other brain region (1.97 ml/min/g for the cerebellum compared to 1.66 ml/min/g for the cerebrum). Absolute increases in blood flow to the lower brainstem were equal to or less than other areas of the brain. We conclude that ponto-medullary blood flow does not increase disproportionate to other areas of the brain during hypercapnia, but some redistribution of CBF does occur in that cerebellar blood flow increased significantly more than the cerebrum, medulla, or pons.

ALTHOUGH INCREASES IN ARTERIAL CO₂ are known to increase both cerebral blood flow (CBF) and respiration, the interaction between these two phenomena has not been adequately studied. For example, stimulation of the medullary respiratory center occurs during hypercapnia resulting in a marked increase in breathing and may increase metabolism in this region of the brain. Since regional CBF is tightly coupled to increases in metabolism, one might expect that the local increase in medullary activity may lead to increases in flow above the level due to the CO₂ stimulus alone. Such a response could lead to a greater increase in blood flow to hindbrain regions than the forebrain during hypercapnia.

Previous studies from other laboratories have only provided indirect support for this hypothesis. Malik et al. stated that the percentage increase in infratentorial blood flow (cerebellum, pons, medulla, and cervical spinal cord) was greater than supratentorial flow in anesthetized dogs mechanically ventilated with 5% CO₂. The findings are somewhat difficult to interpret.
because intracranial pressure (ICP) was also elevated during hypercapnia and since a similar redistribution of CBF occurred when ICP alone was elevated in spontaneously breathing dogs, the effect of hypercapnia alone on the redistribution of CBF is difficult to discern. In another report on newborn puppies, Hernandez et al. reported that asphyxia led to a dramatic redistribution of brain blood flow. In these experiments, blood flow to forebrain structures decreased while blood flow to the hindbrain increased.

Although the above studies suggest that redistribution of CBF is possible during hypercapnia, the alteration of variables other than arterial CO\textsubscript{2} (e.g. ICP or arterial PO\textsubscript{2}) do not permit direct relationships between regional CBF and increased CO\textsubscript{2} to be made. Since elevations in arterial CO\textsubscript{2} are a common occurrence when normal cardiopulmonary function is compromised, the possible redistribution of CBF during hypercapnia is an important problem that deserves further investigation. We chose to study regional CBF in the anesthetized rabbit using the tracer microsphere technique and exercised precautions to alter only arterial CO\textsubscript{2} throughout the experiment.

**Methods**

Seven New Zealand white rabbits weighing 2.9–4.8 kg (mean weight = 4.3 kg) were anesthetized with a IM injection of ketamine and promazine. An endotracheal tube was placed directly into the cervical trachea and anesthesia maintained with methoxyflurane. Galamine triethiodide (Flaxidil, 1 mg per kg body wt) was administered IV to induce muscle relaxation and ventilation maintained with a volume respirator at a respiratory rate of 35/min and a stroke of 20 mls/breath. Supplemental doses of muscle relaxant were given as needed. Rectal temperature was continuously monitored and maintained at the normal body temperature for a rabbit (39°C) by means of a heating pad.

**Measurement of Hemodynamic Variables**

Intravascular catheters were placed in the femoral artery for measurement of arterial blood pressure and heart rate. These variables were monitored using a Statham P231 D pressure transducer and a standard recorder (Gilson Medical Electronics). The arterial catheter also allowed anaerobic collection of heparinized blood samples (0.7 mls in volume) for subsequent measurement of arterial pH, PCO\textsubscript{2} and PO\textsubscript{2} using conventional electrodes (Radiometer). A femoral vein catheter permitted administration of drugs and infusion of a plasma expander (Dextran -40) which was sometimes necessary to maintain normal arterial blood pressures.

Cerebral blood flow was measured by means of tracer microspheres, 15 μ in diameter, injected into the left atrium. The left atrial catheter was placed directly into the heart via a thoracotomy. Between 1.0–1.5 million microspheres, labeled with one of three isotopes (\textsuperscript{57}Co, \textsuperscript{113}Sn, or \textsuperscript{46}Sc) were injected over a 30 second period and the catheter flushed with 2 ml of warmed physiological saline. Precautions were taken to ensure that the microsphere stock solution was adequately mixed and sonicated for several minutes prior to withdrawal of the injectate for subsequent delivery to the animal. Beginning 15 seconds before injection of the microspheres and continuing for 1.5 minutes after injection, a reference arterial blood sample was withdrawn from the femoral artery at a constant rate of 1.57 ml/min. At the end of the experiment the animal was euthanized and the brain removed and fixed in 10% formalin. Following fixation, the radioactivity of the entire brain was measured in the following manner. The brain was dissected into its major anatomical regions, and each region was in turn dissected into smaller tissue samples that were individually placed into a series of test tubes. The height of each tissue sample did not rise more than two cm above the bottom of each tube. The radioactivity of each sample was measured with a multichannel pulse height analyzer (Model NS-710, Tracor Northern) using a three inch well type NaI (Th) crystal.

Energy spans for \textsuperscript{57}Co, \textsuperscript{113}Sn, and \textsuperscript{46}Sc were 57–100 KeV, 205–327 KeV, and 466-857 KeV respectively. Corrections for isotope gamma emission overlap were made using standard methods. Following measurement of the radioactivity of each sample, the activity of those samples from the same brain region were summed to yield the radioactivity of each major brain region. Cerebral blood flow was calculated using the equation CBF = C(t) × Q(a) / C(a), where C(t) is counts per gram of brain tissue, Q(a) is rate of reference arterial blood withdrawal, and C(a) is counts from the reference arterial blood. All blood flows are reported as ml/min/g of tissue. A small sample of microsphere stock solution was also smeared on graph paper and the number of microspheres visually counted with the aid of a microsphere. The radioactivity of the graph paper was then measured and counts per microsphere determined. This procedure allowed the number of microspheres per tissue sample to be calculated to ensure that all tissue samples contained an adequate number of microspheres. Any tissues containing less than 384 microspheres were excluded from the data.

**Experimental Protocol**

Throughout the experiment the rabbits were ventilated with a high oxygen gas mixture to ensure that fluctuations in arterial PO\textsubscript{2} would not alter the variables measured. Cerebral blood flow as well as all other variables were measured three times in each subject. The first measurement was made during normocapnia when two successive arterial blood samples indicated that arterial PCO\textsubscript{2} was near 40 torr. Carbon dioxide was then added to the inspiratory gas and end tidal CO\textsubscript{2} increased from 6 to 10% as monitored with a rapid response CO\textsubscript{2} gas analyzer (Beckman, LB-2 CO\textsubscript{2} gas analyzer). After at least 15 minutes of hypercapnia two successive blood samples were again withdrawn to ensure that the PaCO\textsubscript{2} was near 80 torr. If a steady state was indicated by the measurements of the arterial blood PCO\textsubscript{2}, CBF and all other variables were again measured. Following these measurements, the inspiratory CO\textsubscript{2} was returned to normal levels, and all varia-
TABLE 1  *Arterial Blood Gases, Blood Pressure and Heart Rate during Hypercapnia*

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Hypercapnia</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial PCO₂ (torr)</td>
<td>43.8 (2.3)</td>
<td>79.7 (3.8)†</td>
<td>43.4 (1.2)</td>
</tr>
<tr>
<td>Arterial PO₂ (torr)</td>
<td>382 (28.7)</td>
<td>354 (29.6)</td>
<td>388 (32.7)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.35 (0.02)</td>
<td>7.17 (0.01)†</td>
<td>7.34 (0.03)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>280 (13)</td>
<td>262 (13)</td>
<td>268 (13)</td>
</tr>
<tr>
<td>Arterial blood pressure</td>
<td>70 (5)</td>
<td>71 (5.4)</td>
<td>68 (4.6)</td>
</tr>
</tbody>
</table>

* N = 7 for all means except the measurements during the second control where N = 5. Values in parentheses are standard errors of the mean.
†Significantly (p < 0.05) different from control measurements.

TABLE 2  *Total and Regional CBF during Hypercapnia*

<table>
<thead>
<tr>
<th></th>
<th>Weight (grams)</th>
<th>Control 1</th>
<th>Hypercapnia†</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total brain blood flow (ml/min/g)</td>
<td>10.13</td>
<td>0.77 (0.08)</td>
<td>1.64 (0.21)</td>
<td>0.69 (0.05)</td>
</tr>
<tr>
<td>cerebrum</td>
<td>5.81</td>
<td>0.86 (0.10)</td>
<td>1.66 (0.23)</td>
<td>0.74 (0.06)</td>
</tr>
<tr>
<td>midbrain</td>
<td>1.96</td>
<td>0.68 (0.07)</td>
<td>1.64 (0.23)</td>
<td>0.63 (0.05)</td>
</tr>
<tr>
<td>cerebellum</td>
<td>1.30</td>
<td>0.71 (0.08)</td>
<td>1.97 (0.29)</td>
<td>0.69 (0.03)</td>
</tr>
<tr>
<td>pons</td>
<td>0.41</td>
<td>0.49 (0.05)</td>
<td>1.04 (0.12)</td>
<td>0.51 (0.04)</td>
</tr>
<tr>
<td>medulla</td>
<td>0.65</td>
<td>0.52 (0.04)</td>
<td>1.15 (0.09)</td>
<td>0.53 (0.03)</td>
</tr>
</tbody>
</table>

*Values are means for seven rabbits except for Control 2 which only includes five animals. Blood flow to the medulla and pons during hypercapnia is a mean of six observations in six animals. Numbers in parentheses are standard errors of the mean.
†All values during hypercapnia are significantly (p < 0.05) different from control measurements.

Statistical Analysis

The effect of hypercapnia on arterial blood gas and hemodynamic variables was tested for significance using a 2-way analysis of variance without replication. The two main effects were arterial PCO₂ (normocapnia vs hypercapnia) and animals. Statistical treatment of regional CBF involved a 3-way analysis of variance without replication with the third main factor being the five regions of the brain. An analysis of variance was also used to test for significant differences between the percentage increase in regional blood flow during hypercapnia. If the analysis of variance indicated a significant difference between means, a Student-Newman-Keuls test was used to determine which means differed from one another. A regression of CBF vs arterial PCO₂ was also calculated and tested for significance. For all comparisons, p < 0.05 was considered significant.

Results

Arterial PCO₂ during the initial control period was 43.8 torr and increased to 79.7 torr during hypercapnia (table 1). The increase in PaCO₂ resulted in a decrease in arterial pH of 0.18 units. A high arterial PO₂ (> 350 torr) indicated a hyperoxic state throughout the experiment, while arterial blood pressure and heart rate were unchanged during hypercapnia. The second control measurements made following hypercapnia (Control 2) were not significantly different from the initial baseline measurements (Control 1).

Cerebral blood flow during normocapnia was 0.77 ml/min/g or 7.80 ml/min based on an average brain weight of 10.13 grams (table 2). Blood flow increased an average of 113% during hypercapnia. A regression equation was calculated for the increase in CBF during hypercapnia (fig. 1). Over the PCO₂ range of 35 to 90 torr CBF increased 0.028 ml/min/g for every 1 torr increase in arterial PCO₂. During normocapnia (Control 1) blood flow to the cerebrum was significantly greater than blood flow to the pons or medulla (table 3). The distribution of blood flow to the brain was altered during hypercapnia in that the cerebellum received the greatest flow, followed by the cerebrum and midbrain. Blood flow to the medulla and pons was significantly less than flow to the cerebrum or midbrain. Statistical analysis of the percentage data indicated that the percent increase in blood flow to the cerebellum during hypercapnia was significantly greater than the percent increase in flow to the medulla, pons and cerebrum.

Discussion

The tracer microscope technique is an accepted method for the measurement of both total and regional CBF. We exercised all the necessary precautions in using the technique including the injection of a sufficient number of microspheres to insure an adequate concentration of microspheres in each tissue sample counted. We also chose the left atrium for the site of injection to insure adequate mixing of microspheres with the blood prior to distribution via the systemic flow.
Table 3  Regional Distribution of CBF during Normocapnia and Hypercapnia*

<table>
<thead>
<tr>
<th></th>
<th>Normocapnia</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrum</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>Percent of total CBF†</td>
<td>64%</td>
<td>12%</td>
</tr>
<tr>
<td>Percent of total CBF†</td>
<td>15%</td>
<td>58%</td>
</tr>
<tr>
<td>Δ increase in flow (ml/min/g)§</td>
<td>1.26</td>
<td>0.80</td>
</tr>
<tr>
<td>Percent increase in flow during hypercapnia§</td>
<td>177%</td>
<td>93%</td>
</tr>
</tbody>
</table>

*N = 7, A common line indicates no significant difference in blood flow as measured in ml/min/g.
†Percent of total CBF calculated as regional flow (ml/min) / total brain blood flow (ml/min) × 100.
‡Δ increase in flow calculated as regional blood flow (ml/min/g) during hypercapnia - regional blood flow (ml/min/g) during control.
§Percent increase in flow during hypercapnia = (regional blood flow (ml/min/g) during hypercapnia - regional blood flow (ml/min/g) during control 1) / regional blood flow during control 1 × 100.

We attempted to maintain all variables that influence CBF (other than arterial PCO2) constant throughout the experiment to allow direct interpretation of the data. Arterial blood pressure was maintained at 70 mm Hg during all measurements of CBF. Thorocotomies in the rabbit appear to lead to reductions in arterial blood pressure since the normal blood pressure in awake rabbits has been reported to be near 90 mm Hg. Heistad et al. maintained arterial blood pressure constant by partially occluding the abdominal aorta, while we have chosen to administer a plasma expander (Dextran -40) to prevent any further hypotension. No animals were included in this study with a blood pressure less than 60 mm Hg which seems to be well within the limits of autoregulation of CBF.

Although the baseline blood flows in this study are slightly higher than Heistad et al. report for the anesthetized rabbit, the data are somewhat difficult to compare due to the differences in anesthesia and the lower baseline arterial PCO2 in the former study. Blood flow to the cerebellum and hindbrain as reported by Sadoshima et al. agree well with the present data. In addition, the slope of the regression line (PaCO2 vs CBF) does not appear to be significantly different from previous reports on anesthetized dogs and goats.

The distribution of CBF during normocapnia appears qualitatively similar to previous reports on the rabbit and other animal species. Forebrain regions such as the cerebrum receive greater blood flow per gram of tissue than do hindbrain regions (pons, medulla) at normal levels of arterial CO2.

The redistribution of organ blood flow in response to a chosen stimulus is most apparent when a directional difference in the vascular response is observed in separate regions of the organ (i.e. increase in flow in one region vs a decrease in flow in another region). The more common finding, however, is a difference in the magnitude of the response (i.e. a greater increase in one region vs another). Various calculations can be utilized to demonstrate this response, four of which are presented in table 3.
Although the percent increase in flow to the pons and medulla during hypercapnia was greater than the cerebrum, we do not believe that this indicates a differential response to hypercapnia in these brainstem regions. Rather, blood flow to the medulla and pons was less than blood flow to any other brain region during both normocapnia and hypercapnia and the percent of total CBF to these brain regions was not significantly altered during hypercapnia. Additionally, the absolute increase in blood flow was not significantly different than the increase in flow to the cerebrum or midbrain. We conclude that the increase in medullary and pontine blood flow during hypercapnia is similar to other brain regions and that the greater percentage increase in flow simply reflects a lower initial (normocapnic) blood flow to these brainstem regions.

At the selected level of hypercapnia (arterial PCO$_2$ near 80 torr), medullary metabolism should have been increased, but the increase may have been too localized to increase flow to the entire medulla. To examine blood flow more specifically would require the injection of a quantity of microspheres that would occlude too many vessels and anatomical isolation of the respiratory control center would be difficult. The present data simply indicate that increases in medullary metabolism during hypercapnia are not sufficient to cause a disproportionate increase in blood flow to the medulla.

The more interesting finding, however, was that blood flow to the cerebellum during hypercapnia was significantly greater than blood flow to any other brain region. This distribution of flow is strikingly different from that observed during normocapnia where cerebellar, cerebral and midbrain flow did not differ from one another. Calculation of the percent of total CBF distributed to the cerebellum during normocapnia vs hypercapnia, absolute increases in flow per gram of tissue, and percent increase in flow during hypercapnia are consistent with the statement that cerebellar blood flow increased more than other brain regions.

Redistribution of CBF has been reported to occur during other experimental conditions. The greater increase in infratentorial blood flow compared to supratentorial flow during intracranial hypertension in spontaneously breathing dogs reported by Malik et al. has recently been supported by Sadoshima et al. in anesthetized rabbits. Redistribution of CBF has been reported to occur during both severe hypotension and exercise. Marcus et al. indicated that medullary blood flow decreased less than blood flow to other regions of the brain during severe hypotension in awake dogs. Although no mechanism for this redistribution of flow was offered, the authors commented on the protective nature of this response for vital centers in the medulla. Gross et al. reported that during moderate exercise of awake dogs, total CBF remained unchanged, but blood flow to the motor sensory cortex and the cerebellum increased, probably due to increased metabolic activity in these regions of the brain.

We can only speculate as to why cerebellar blood flow increased significantly more than blood flow to the cerebrum or hindbrain during hypercapnia. Malik et al. suggested that a possible explanation for the greater increase in blood flow to infratentorial regions compared to supratentorial regions in spontaneously breathing dogs with elevated ICP may be ICP gradients between various brain regions. Increases in cerebrospinal fluid pressure are known to occur during hypercapnia, but the time course and possible heterogeneity of this pressure increase has not been adequately studied. It is difficult to envision, however, why ICP would increase in a heterogeneous manner during hypercapnia in the normal animal, and the involvement of ICP changes in this response seems unlikely.

Hernandez et al. has outlined a mechanism to explain the non-uniform changes in CBF observed during asphyxia in the newborn puppy. These investigators suggest that sympathetic stimulation of extraparenchymal cerebral vessels may attenuate the vasodilatory effects of asphyxia. Since forebrain blood vessels possess a denser adrenergic innervation than hindbrain regions, a differential response to asphyxia is possible. Although this mechanism may contribute to our findings in the adult rabbit, the hypothesis remains speculative and has not been supported by any direct investigations.

Another possible mechanism for the non-uniform increase in CBF observed in the rabbit during hypercapnia may be differential concentrations of grey vs white matter in various brain regions. Utilizing data recently presented by Busija and Heistad one can calculate the relative increase in blood flow as measured with tracer microspheres to grey vs white matter in the anesthetized cat during hypercapnia. Such calculations indicate that the relative increase in flow to cerebral grey matter was approximately 2.5 times the increase in flow to cerebral white matter. It has also been reported that myelin concentrations in the cerebellum are significantly less than brainstem regions and are less than or equal to the cerebral myelin concentrations. If low concentrations of myelin in the cerebellum can be assumed to reflect high concentrations of grey matter this may suggest that the large increase in cerebellar flow may be due in part to the relative amounts of grey vs white matter in this region of the brain. Further investigations into the distribution of grey vs white matter in the brain and the reactivity of these two tissues to vascular stimuli would appear warranted.

References

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J A Orr, R C DeSoignie, L C Wagerle and D B Fraser

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