Mechanism of Cerebral Blood Flow Augmentation by Hemodilution in Rabbits

Kazuyoshi Korosue, MD, and Roberto C. Heros, MD

Background and Purpose: Hemodilution is known to increase cerebral blood flow, but it is not known whether the increase in flow is a direct result of a decrease in viscosity or whether it may be due to compensatory vasodilatation in response to the decrease in oxygen carrying capacity that results from hemodilution. This study is designed to investigate this question.

Methods: Changes in regional cerebral blood flow were studied in normal and ischemic brains of 15 and 18 rabbits, respectively. In one group of rabbits graded hemodilution was used to reduce arterial oxygen content progressively in stages; in the second group the arterial oxygen content was reduced in similar stages by progressively larger reductions in the concentration of inspired oxygen (hypoxic hypoxia). In the ischemic animals focal ischemia was produced by embolic occlusion of the right middle cerebral artery.

Results: In the normal rabbits, hypoxic hypoxia and hemodilution resulted in similar progressive increases in cerebral blood flow as arterial oxygen content fell. In the ischemic animals, there was a significant fall in cerebral blood flow in the ischemic region in all groups after arterial occlusion. Hemodilution resulted in a progressive increase in cerebral blood flow in both ischemic and nonischemic regions. With hypoxic hypoxia, however, cerebral blood flow in the ischemic region showed no increase or a slight decrease.

Conclusions: Even though hypoxic hypoxia results in a marked increase in cerebral blood flow in normal brain, it does not significantly change cerebral blood flow in ischemic brain. In contrast, hemodilution resulting in a comparable degree of hypoxemia is capable of significantly increasing cerebral blood flow in ischemic brain. Therefore, the mechanism of blood flow augmentation by hemodilution in ischemic brain is probably related to a direct hemorheologic effect rather than to the resulting hypoxemia. (Stroke 1992;23:1487–1493)

KEY WORDS • cerebral blood flow • cerebral ischemia • hemodilution • rabbits
steps until it reached 30% of the baseline value. As a result, there were five stages in which \( \text{CaO}_2 \) was approximately 85%, 70%, 55%, 40%, and 30% of the baseline value. The \( \text{rCBF} \), \( \text{MABP} \), arterial blood gases, \( \text{Hb} \) concentration, and \( \% \text{O}_2 \) Hb were measured 15 minutes after each stage of hemodilution.

In the hypoxic hypoxia subgroup, \( \text{CaO}_2 \) was decreased serially to similar degrees as in the hemodilution subgroup by reducing the inhaled \( \text{O}_2 \) concentration and increasing the inhaled nitrogen concentration in progressively larger steps. At severe hypoxia a fall in \( \text{Paco}_2 \) was avoided by adding \( \text{CO}_2 \) to the inspired gas mixture or by adjusting ventilation rate and/or volume. The \( \text{rCBF} \), \( \text{MABP} \), arterial blood gases, \( \text{Hb} \) concentration, and \( \% \text{O}_2 \) Hb were measured 5 minutes after the induction of hypoxia in each \( \text{CaO}_2 \) stage. To maintain a constant cardiovascular function, after each \( \text{rCBF} \) measurement the inhaled \( \text{O}_2 \) concentration was returned to the baseline level for 30 minutes.

We used 18 rabbits in the ischemic experiments; the animals were divided into three subgroups: control (\( n=6 \)), hemodilution (\( n=7 \)), and hypoxic hypoxia (\( n=5 \)). In the control subgroup, after the second measurement of baseline \( \text{rCBF} \), the right middle cerebral artery (MCA) was occluded by an embolic technique to be described. The above-mentioned parameters were measured every hour for 8 hours after MCA occlusion. In the hemodilution and hypoxic hypoxia subgroups, stages of graded reduction in \( \text{CaO}_2 \) were achieved in the same fashion as in the nonischemic experiments after the second baseline measurement and MCA occlusion. All the physiological parameters were also measured in the same manner as in the nonischemic experiments.

Anesthesia was induced with 15 mg/kg thiamylal sodium and was maintained with 2% halothane in 70% \( \text{N}_2 \)/30% \( \text{O}_2 \) during the surgical procedure. A tracheostomy was performed, the rabbit was immobilized with 0.3 mg/kg pancuronium bromide, and mechanical ventilation was continued with a Harvard respirator (South Natick, Mass.) while mechanically ventilated on 70% \( \text{N}_2 \)/30% \( \text{O}_2 \). We believed that to obtain stable \( \text{CBF} \) values we had to discontinue halothane because this agent is known to increase \( \text{CBF} \) initially, with the effect tapering off in an unpredictable manner over time. To avoid painful stimulation of the rabbit, no further surgical manipulations were done after halothane was discontinued.

A silicone embolus, 0.4 mm in diameter and 6 mm long, was injected with 0.5 ml of 0.9% saline through the catheter placed in the ICA. At the end of the experiment, brains of the ischemic control subgroup were cut coronally at the level of the coronal suture and were stained with 2% 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) for 30 minutes at 37°C.

Electrodes for \( \text{rCBF} \) measurement were placed the day before the experiment under halothane anesthesia. The electrodes were made of 90% platinum and 10% iridium, 150 \( \mu \text{m} \) in diameter, and were coated with Teflon except for 1 mm of the bare tip, which was sharpened electrochemically. Electrodes were placed into the cortex through bilateral burr holes 6 mm lateral to the midline at the level of the coronal suture. Burr holes were sealed with agar-agar in 0.9% saline, and the electrodes were fixed to the skull with dental cement. The Ag-AgCl reference electrode was placed subcutaneously in the neck. The platinum electrode on the right side measured \( \text{rCBF} \) in the presumed area of ischemic penumbra in the ischemic experiments.

The hydrogen clearance technique was used to measure \( \text{rCBF} \). The electrodes were polarized at 0.36 V and \( \text{H}_2 \) was added to the inspired gas mixture to get an approximately 5% \( \text{H}_2 \) concentration. After 5 minutes of inhalation, \( \text{H}_2 \) was discontinued and the hydrogen washout curves were recorded. The \( \text{rCBF} \) at each electrode was calculated from the 2-minute hydrogen clearance using the initial slope method.

Where applicable, subgroup means were compared and contrasted by analysis of variance and the unpaired \( t \) test. The changes in physiological parameters within subgroups were analyzed by the paired \( t \) test. Curves were fit by the least-squares method; goodness of fit was judged on the basis of the correlation coefficient and the significance of regression tested by analysis of variance. Slopes and intercepts were compared using a large-sample \( z \) test for parallelism and common intercept. A probability of less than 0.05 was considered significant.

**Results**

While mechanically ventilated on 70% \( \text{N}_2 \)/30% \( \text{O}_2 \), there were no differences in \( \text{Paco}_2 \), \( \text{Hb} \) concentration, \( \text{CaO}_2 \), \( \text{Paco}_2 \), and \( \text{MABP} \) among the nonischemic subgroups (Table 1). Mean±SEM baseline \( \text{rCBF} \) of the control subgroup (35.0±2.4 ml/100 g/min) was not different from that of the hemodilution or hypoxic hypoxia subgroups (38.4±3.2 and 34.5±2.4 ml/100 g/min, respectively). In the control rabbits, all parameters remained at constant values for 10 hours (Figure 1). In both the hemodilution and hypoxic hypoxia subgroups, \( \text{CaO}_2 \) decreased without significant changes in \( \text{Paco}_2 \) or \( \text{MABP} \) (Table 1). When \( \text{rCBF} \) was plotted as a function of \( \text{CaO}_2 \) (Figure 2) there was a clear relation between \( \text{rCBF} \) expressed as percent of the baseline value and \( \text{CaO}_2 \) in both the hemodilution and hypoxic hypoxia subgroups. At a \( \text{CaO}_2 \) of 4–6 ml/dl, \( \text{rCBF} \) increased to 198% of the baseline value in the hemodilution subgroup and to 209% of baseline in the hypoxic hypoxia subgroup. Comparison of the regression lines for the hemodilution and hypoxic hypoxia subgroups showed no difference between the slopes or intercepts (hemodilution versus hypoxic: slope, -7.4 versus -7.2 and intercept, 212.7 versus 214.7).

In the ischemic group, postmortem examination revealed misplacement of the silicone embolus into the ICA in one control and two hemodilution rabbits. These rabbits were excluded from the study, and the data from...
TABLE 1. Changes in MABP and Paco2 in Rabbits Without Cerebral Ischemia During Hypoxia

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>CaO2 (ml/dl)</th>
<th>14</th>
<th>12-14</th>
<th>10-12</th>
<th>8-10</th>
<th>6-8</th>
<th>&lt;6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td>102±10.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paco2</td>
<td>32.5±4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodilution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td>105±14.2</td>
<td>107±11.8</td>
<td>103±13.4</td>
<td>100±14.5</td>
<td>108±11.1</td>
<td>106±14.5</td>
<td></td>
</tr>
<tr>
<td>Paco2</td>
<td>31.4±3.4</td>
<td>32.4±2.1</td>
<td>34.2±4.4</td>
<td>33.2±3.5</td>
<td>32.4±2.8</td>
<td>33.5±2.9</td>
<td></td>
</tr>
<tr>
<td>Hypoxic hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td>108±11.3</td>
<td>105±18.8</td>
<td>108±15.5</td>
<td>106±13.6</td>
<td>110±19.8</td>
<td>106±11.9</td>
<td></td>
</tr>
<tr>
<td>Paco2</td>
<td>34.5±4.4</td>
<td>35.5±2.5</td>
<td>36.7±2.1</td>
<td>34.5±5.7</td>
<td>31.1±5.6</td>
<td>32.4±4.9</td>
<td></td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure; Paco2, arterial pressure of carbon dioxide; CaO2, arterial oxygen content.

Values are mean±SEM mm Hg in five animals.

Korosue and Hews  
Mechanism of CBF Increase With Hemodilution  
1489

the remaining 15 rabbits (five in each subgroup) were analyzed.

There were no differences in baseline values of all the above-mentioned parameters, including rCBF, among the ischemic subgroups (Table 2). In the control subgroup rCBF on the ischemic side decreased significantly from 37.2±4.8 to 14.4±6.4 ml/100 g/min after MCA occlusion, and the values remained stable thereafter for 8 hours (Figure 3). MABP, PaO2, and Paco2 remained constant throughout the experiment (Figure 3). TTC staining showed an area of presumed infarction in the caudate nucleus, the putamen, and a small area of the cerebral cortex in the frontoparietal region laterally. In two of the five control rabbits, the platinum electrodes in the ischemic side were located outside but very close to the infarcted area; in the rest, the electrodes were located at the edge of the infarct.

After embolization, rCBF decreased significantly from 35.2±4.0 to 20.1±7.2 ml/100 g/min in the hemodilution subgroup and from 36.3±5.8 to 19.2±6.4 ml/100 g/min in the hypoxic hypoxia subgroup. In the hemodi-
lution subgroup, rCBF related inversely to CaO₂ in both ischemic and nonischemic areas, although the increase in rCBF was less in the ischemic area (Figure 4). Slopes of the regression lines were -6.4 and -3.0 and intercepts were 205.7 and 150.4 in the nonischemic and ischemic areas, respectively. At a CaO₂ of 4-6 ml/dl, rCBF increased to 137% of the baseline value in the ischemic area and to 173% in the nonischemic area. In the hypoxic hypoxia subgroup, rCBF in the nonischemic area also showed an inverse correlation to CaO₂ and increased to 180% of the baseline value at a CaO₂ of 4-6 ml/dl (Figure 4). On the other hand, rCBF in the ischemic region showed no increase or a slight decrease, especially at low CaO₂ (Figure 4). Slope and intercept of the regression line in the nonischemic area were -7.1 and 211.7 while values in the ischemic area were 2.0 and 72.2, respectively. The regression line for the ischemic side in the hemodilution subgroup was significantly different from that for the ischemic side in the hypoxic hypoxia subgroup (p<0.01 for both slope and intercept). In other words, rCBF in the ischemic region responded very differently to hypoxic hypoxia than to hemodilution, with no change or a slight decrease during hypoxic hypoxia and a substantial increase during hemodilution, as expected. This dissociation could be based on the local difference of metabolic activity or acid–base relations, which could affect regional vascular activity.

Because hemodilution decreases both CaO₂ and blood viscosity, it has been impossible to identify an independent effect of blood viscosity on CBF after hemodilution. Several previous studies have tried to separate these two factors by means of plasma exchange using low-viscosity plasma substrates, carbon monoxide hypoxia, or blood exchange with methemoglobin-containing erythrocytes. These studies came to the same conclusion, that the fall of CaO₂, not blood viscosity, was the primary mediator of the increase in CBF with hemodilution. However, it should be noted that these studies were carried out in normal, rather than ischemic, tissue.

In the present study, hypoxia was induced by either hemodilution or reduction of the inspired O₂ concentration. CaO₂ depends mainly on the volume of oxygen bound to Hb, together with a very small amount of oxygen dissolved in the plasma. Because the volume of oxygen bound to Hb is determined by the Hb concentration and %O₂ Hb, the major factors responsible for changes in CaO₂ are the Hb concentration and %O₂ Hb. Hemodilution decreases CaO₂ by decreasing the Hb concentration, which also results in a decrease in blood viscosity. On the other hand, hypoxic hypoxia reduces CaO₂ by lowering %O₂ Hb, but because the Hb concentration does not change, viscosity remains constant. The present study demonstrated that the CBF response to CaO₂ was exactly the same between the subgroup with anemic hypoxia (hemodilution) and the subgroup with hypoxic hypoxia and that both types of hypoxia caused a linear increase in rCBF throughout the CaO₂ range studied. Jones et al observed a similar CBF response in newborn lambs in which CaO₂ was varied by changing the inspired O₂ concentration at a high or low Hct in the same lamb. The authors concluded that a tight coupling between CBF and CaO₂ maintained cerebral O₂ transport relatively constant.

It is important to emphasize again that all the results described above apply to normal cerebral tissue with presumably intact autoregulation. Gaechtgens et al reported a physiological hemodilution that causes a progressive decline of Hct as blood reaches the smaller vessels and finally results in a capillary Hct value of one third that in the large arteries. Based on this phenom-

### Table 2. Changes in MABP and PaCO₂ in Rabbits With Cerebral Ischemia During Hypoxia

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>CaO₂ (ml/dl)</th>
<th>14</th>
<th>12-14</th>
<th>10-12</th>
<th>8-10</th>
<th>6-8</th>
<th>&lt;6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td></td>
<td>105±6.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PaCO₂</td>
<td></td>
<td>35.6±4.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hemodilution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td></td>
<td>102±11.9</td>
<td>105±18.3</td>
<td>104±12.2</td>
<td>104±15.5</td>
<td>109±12.1</td>
<td>109±15.3</td>
</tr>
<tr>
<td>PaCO₂</td>
<td></td>
<td>33.5±4.4</td>
<td>35.4±4.6</td>
<td>34.5±3.3</td>
<td>34.8±4.1</td>
<td>36.6±6.8</td>
<td>33.5±2.9</td>
</tr>
<tr>
<td><strong>Hypoxic hypoxia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td></td>
<td>106±14.4</td>
<td>104±15.2</td>
<td>108±16.5</td>
<td>109±14.6</td>
<td>109±16.6</td>
<td>108±16.9</td>
</tr>
<tr>
<td>PaCO₂</td>
<td></td>
<td>35.7±2.3</td>
<td>35.5±2.3</td>
<td>36.9±3.6</td>
<td>34.4±4.1</td>
<td>32.1±4.8</td>
<td>32.4±5.7</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure; PaCO₂, arterial pressure of carbon dioxide; CaO₂, arterial oxygen content. Values are mean±SEM mm Hg in five animals.
Korosue and Hems
Mechanism of CBF Increase With Hemodilution

Regional Cerebral Blood Flow

Hemodilution (Anemic Hypoxia)

Mean Arterial Blood Pressure

PaCO₂

FIGURE 3. Plots of changes in regional cerebral blood flow (top), mean arterial blood pressure (center), and arterial pressure of carbon dioxide (PaCO₂, bottom) in control rabbits with cerebral ischemia. Each point represents mean in five animals. Arrow indicates occlusion of middle cerebral artery.

FIGURE 4. Plots of relations between arterial oxygen content (CaO₂) and regional cerebral blood flow (CBF%) in ischemic (o) and nonischemic (□) areas of rabbits with cerebral ischemia. For hemodilution, ischemic side: CBF% = −3 × CaO₂ + 150.4, r = 0.62, p < 0.01; nonischemic side: CBF% = −6.4 × CaO₂ + 205.7, r = 0.91, p < 0.01; for hypoxic hypoxia, ischemic side: CBF% = 2 × CaO₂ + 72.2, r = 0.63, p < 0.01; nonischemic side: CBF% = −7.1 × CaO₂ + 211.7, r = 0.89, p < 0.01.

phenomenon, Wood et al.⁷ suggested that under normal conditions the Hct effect on viscosity in the microcirculation is almost negligible. In ischemic tissue, however, local coupling of CBF to tissue demands of O₂ has been disrupted and resistance vessels are already maximally dilated due to a deficiency of O₂ delivery.¹⁶⁻¹⁸⁻²⁰ CBF may then passively follow changes in perfusion pressure, which is very low in regions of ischemia. Under such circumstances physiological hemodilution, which is dependent on vasoconstriction, is no longer possible and Hct in the microcirculation increases abruptly to the level in large vessels, with a resulting dramatic increase in blood viscosity. This will tend to slow blood flow even further and to enhance the formation of microaggregates and thrombi in the ischemic region.²¹⁻²³ Thus, blood rheology may play a pivotal role in determining cerebral perfusion in ischemic regions where CBF may be significantly improved by hemodilution. Indeed, many studies have shown a significant effect of hemodilution on CBF in ischemic brain in both experimental and clinical settings.¹²⁻¹⁷⁻²⁴ Our results in the ischemic experiments confirm this hypothesis and demonstrate a significant increase in CBF in ischemic brain by graded hemodilution. In contrast, graded hypoxic hypoxia failed to increase CBF in the ischemic area. Except for viscosity, other physiological parameters (including PaCO₂) were not significantly different between the subgroups. Therefore one may conclude that, in contrast with the changes in CBF observed in normal brain, the changes in CBF in ischemic brain are mainly a rheological response to the alterations in blood viscosity, rather than a physiological response to a decrease in CaO₂.

Even though in this study hemodilution resulted in a significant increase in CBF in the ischemic region, it is not clear whether the increase in CBF overrides the decrease in CaO₂ and results in a net increase in O₂ delivery to ischemic brain. One way to investigate tissue O₂ delivery is to calculate O₂ delivery using rCBF and regional CaO₂. Our electrodes, with a spatial resolution of about 0.5 mm, can detect only the average rCBF of the several arterioles and capillaries within that volume.
of tissue. In addition, to calculate regional CaO₂ it is essential to use Hct in the microcirculation of the region of interest, instead of Hct in large systemic vessels. Such measurement, however, is hardly possible. A more direct way to study tissue O₂ delivery is to measure tissue O₂ tension directly with microelectrodes small enough to detect the O₂ tension between capillaries. Using multiple electrodes that allowed the construction of a histogram of tissue oxygenation to assess the average supply of O₂ to cerebral tissue, Chan et al. investigated the effect of isovolemic hemodilution on local tissue O₂ tension and the power of the electrocorticogram (ECOＧ) in normal and ischemic brain. As expected, after MCA occlusion local tissue O₂ tension and ECoG markedly decreased. Subsequent isovolemic hemodilution did not cause any change of tissue O₂ tension or ECoG in normal or ischemic brain until Hct reached <20%. Unfortunately, this report did not elaborate on the severity of ischemia or on the relation between the ischemic lesion and the position of the electrodes. Therefore, it is not clear whether tissue O₂ tension and ECoG were measured in the ischemic core or in the periphery of the ischemic region.

In summary, the present study has shown that in normal brain CaO₂ is a major determinant of CBF. In ischemic brain, however, a major coupling of CBF to tissue demands for O₂ is disrupted and blood viscosity becomes a major factor determining CBF.

References

Editorial Comment

Korosue and Heros have attempted to answer the question of whether the increase in cerebral blood flow (CBF) with hemodilution is due to diminished O₂ content with ensuing vasodilation or due to the decrease in blood viscosity. The new aspect of this work is that hypoxic hypoxia and isovolemic anemia were compared in normal and in ischemic brain. Although ideally a control group of animals receiving isovolemic transfusion with whole blood without anticoagulants should have been included, I still agree with the authors that their data convincingly show that decreased viscosity is the mechanism leading to increased CBF with hemodilution. In the important paper by Hudak and colleagues, our own finding of pial arteriolar constriction with hemodilution was confirmed, while despite this constriction those authors found an increase in CBF. Hudak et al. could not discriminate between a viscosity effect or vasodilation in the larger arteries, but we have recently shown that the diameter of the basilar artery hardly changes with either hemodilution or hemoconcentration (if anything, the artery slightly constricts with hemodilution). Taken altogether, in my mind the influence of blood viscosity in CBF is well established.
Mechanism of cerebral blood flow augmentation by hemodilution in rabbits.
K Korosue and R C Heros

Stroke. 1992;23:1487-1492
doi: 10.1161/01.STR.23.10.1487

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/10/1487

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/