Effect of hemodilution on RBC velocity, supply rate, and hematocrit in the cerebral capillary network

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Hudetz, Antal G., James D. Wood, Bharat B. Biswal, Ines Krolo, and John P. Kampine. Effect of hemodilution on RBC velocity, supply rate, and hematocrit in the cerebral capillary network. J. Appl. Physiol. 87(2): 505–509, 1999.—The effect of isovolemic hemodilution on the circulation of red blood cells (RBCs) in the cerebrocortical capillary network was studied by intravital videomicroscopy with use of a closed-cranial-window technique in the rat. Velocity and supply rate of RBCs were measured by tracking the movement and counting the number of fluorescently labeled cells. Arterial blood was withdrawn in increments of 2 ml and replaced by serum albumin. Arterial blood pressure was maintained constant with an infusion of methoxamine. Both velocity and supply rate of RBCs increased, by approximately equal amounts, as arterial hematocrit was reduced from 44 to 15%. The maximum increase in RBC velocity was 4.6 and in RBC supply rate was 5.2 times the baseline value. Calculated lineal density of RBC, an index of capillary hematocrit, did not change with hemodilution. The results suggest that RBC flow and oxygen supply in the cerebral capillary network are maintained during isovolemic hemodilution. The “optimal hematocrit” is as low as 15%.

cerebral blood flow; microcirculation; anemia; plasma skimming; oxygen transport; red blood cell

THE ROBUST HYPEREMIC EFFECT of isovolemic hemodilution in the cerebral circulation is well known (2, 5, 10, 13, 19, 23, 28, 30). Cerebral blood flow may increase as high as eightfold of its resting level when arterial hematocrit is reduced below 10% (2). With moderate reductions in arterial hematocrit, oxygen supply to tissue is thought to improve because the increase in blood flow exceeds the decrease in oxygen-carrying capacity of blood. The existence of an “optimal hematocrit” in the cerebral circulation has been proposed (5). According to this concept, maximum oxygen delivery to tissue is attained at an arterial hematocrit of ~30%. Oxygen delivery would fall when hematocrit is further reduced.

Despite the general acceptance of these concepts, the effect of isovolemic hemodilution on cerebral capillary perfusion, particularly by the oxygen-carrying red blood cell (RBC), has not been directly examined. This is important because in the microcirculation RBC flow may dissociate from blood flow or plasma flow because of a change in plasma skimming and the Fahraeus effect (21, 22). Thus the change in supply rate of RBCs may be different from that predicted by the measurements of regional cerebral blood flow.

Previously, Rosenblum (23) studied RBC velocity in pial arterioles and venules and plasma transit time through intracortical vessels of the mouse brain and found that RBC velocity increased but plasma velocity decreased during isovolemic anemia. When systemic hematocrit was increased by various means, different patterns of changes in RBC flow relative to plasma flow were observed (25). The supply rate of RBCs through intracortical capillaries per se was not assessed. Recently, Todd et al. (28) measured intracortical RBC and plasma velocities by radioactive labeling and predicted a large decrease in calculated microvascular hematocrit during isovolemic hemodilution. This finding appears to be at variance with the majority of reports for the peripheral microcirculation (14, 16–18). These results underline the need to reexamine cerebral capillary perfusion patterns during hemodilution by direct, microscopic visualization.

In the present study, intravital fluorescence videomicroscopy was used to determine the effect of isovolemic hemodilution on RBC velocity, supply rate, and content in the intracortical capillary network. We hoped to answer the following questions. 1) How much does the velocity of RBCs in cerebral capillaries increase during isovolemic hemodilution? 2) Does RBC supply rate increase to the same extent as RBC velocity? 3) Does capillary hematocrit, or the lineal density of RBC in capillaries, decrease when arterial hematocrit is reduced?

METHODS

Experimental preparation. The experiments were performed on four male Sprague-Dawley rats weighing 327–445 g. The animals were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized, and femoral arterial and venous lines were placed for the measurement of arterial blood pressure and blood gases, for blood withdrawal, and for the infusion of drugs. Anesthesia was maintained with pentobarbital sodium infusion at 7–10 mg/h. The head was secured in a stereotaxic apparatus, and a closed cranial window was installed over the right parietal cortex by using a technique previously described (7). The window was equipped with ports for superfusion with artificial cerebrospinal fluid at 37°C and for the measurement of intracranial pressure. The latter was adjusted to 5–10 mmHg. After surgery, the animals were transferred to the intravital microscope, paralyzed with gallamine (80 mg), and artificially ventilated with a mixture of 30% O2–70% N2. Arterial blood pressure, end-tidal CO2 (EtCO2), and inspired oxygen concentrations were continuously monitored (POET II, Criticare Systems). The rate
and volume of ventilation were adjusted to maintain the 
$ETCO_2$ at 35 Torr. In each animal, before the beginning of the 
hemodilution protocol, the $ETCO_2$ setting was adjusted, as
necessary, to obtain normal arterial CO$_2$ levels. The arte-
rial blood gases in the control state were as follows: $PO_2 =
131 \pm 12$ Torr, $PCO_2 = 38 \pm 4$ Torr, and $pH = 7.35 \pm
0.03$.

Videomicroscopy of the capillary circulation. To facilitate
the visualization and measurement of flow in the microcircu-
lation, RBCs were labeled in vitro with FITC and injected
intravenously in tracer quantities into the circulation of the
experimental animal (7). With the use of epifluorescence and
a $\times 40$ objective, the passage of labeled cells through intracor-
tical capillaries 50–70 $\mu$m below surface could be visualized
in real time and video recorded. The optical magnification
was $\times 125$, resulting in a field size of 400 by 300 $\mu$m. Although
the capillary walls themselves were not visible, capillaries
could be identified by the presence of single-file RBC flow
(impling vessel diameter of <5 $\mu$m) and by the tortuous and
branching pathway of cells. In some of the experiments, the
interconnected network of capillaries was reconstructed by
following the course of several cells (8). To minimize the
exposure of the preparation to light, a 50% neutral-density
filter was used and continuous illumination was limited to
1-min-long periods while the circulation was video recorded.
In addition, a heat filter and a 455-nm high-pass filter were
used to block infrared and ultraviolet irradiation of the tissue.

Experimental protocol. Graded isovolemic hemodilution
was produced by the repeated withdrawal of 2 ml of blood,
which were immediately replaced by the same volume of
serum albumin. A sample of 100 $\mu$l blood was used for
hematocrit determination. In two of these and additional
three parallel experiments, arterial blood gases were deter-
mined after each hemodilution step. To support the arterial
blood pressure, the $\alpha_1$-agonist methoxamine was infused
intravenously at a rate between 1 and 15 mg·kg$^{-1}$·h$^{-1}$ as in
prior studies (6). Intravenous $\alpha_1$-selective adrenergic agonists
have been reported to exert no direct effect on the cerebral
vasculature (27). The rate of infusion of methoxamine was
increased after each hemodilution step as required to main-
tain mean arterial pressure (MAP) at baseline level. The
infusion of methoxamine at the lowest rate was usually
started after the third or fourth hemodilution step. In each
animal the microcirculation was video recorded in the control
state (before hemodilution) and 5–7 min after the completion
of each hemodilution step. This time frame was found to be
sufficient to stabilize MAP at the control level and short
enough to preserve cardiovascular stability of the preparation
for the duration of the experiment.

Data analysis. The velocity and supply rate of RBCs were
measured in individual capillaries during video playback.
Figure 1 shows video images obtained at different flow rates
in a hemodilution experiment, as an example. The velocity
was measured by frame-to-frame tracking of labeled RBC by
using an imaging system developed in our laboratory (8). In
each experiment, four to eight capillaries were chosen for
measurement. RBC velocity was measured at 3- to 5-s
intervals, and the obtained velocities were averaged over the
1-min recording period. RBC supply rate, defined as the
number of labeled cells passing through a capillary per
minute, was measured by visual counting over the same
period. RBC supply rate is identical to what was formerly
called RBC flux (7, 12). Lineal cell density (the number of
labeled RBC per millimeter length of capillary) was calcu-
lated as the ratio of RBC supply rate to RBC velocity in the
same capillary. Statistical analysis of the data was performed
by using linear regression and repeated-measures ANOVA.

Fig. 1. Fluorescently labeled red blood cells (RBC) passing through
subsurface cortical capillaries at slow (A), intermediate (B), and fast
(C) flow. Cells appear as bright spots when moving slowly and as
fainter streaks when moving fast (see arrows). Capillaries them-
selves are not visible; only the labeled cells passing through them are.
Larger bright objects are from cells in larger surface vessels that are
out of focus. Leading edge of several cells was tracked frame to frame
to obtain RBC velocity in each capillary. Images were reproduced by
using a video printer from SVHS videotape without image enhance-
ment. Printed field size is 300 by 400 $\mu$m.
RESULTS

Average baseline RBC velocity in all measured capillaries was 0.65 ± 0.04 mm/s. Arterial hematocrit was 43 ± 1%, and MAP was 123 ± 5 mmHg. Stepwise isovolemic hemodilution produced consistent increase in RBC velocity in each capillary. Figure 2 illustrates the average change in RBC velocity in each experiment. A significant increase (P < 0.005) in velocity was obtained at and below the hematocrit of 32%. At the last hemodilution step, RBC velocity reached a level 4.6 times the baseline.

Figure 2 also shows that MAP was well maintained during the experimental protocol. MAP after the last step of hemodilution was 111 ± 6 mmHg, not significantly different from control. Arterial P O2 was also within physiological limits at 114 ± 31 Torr. RBC supply rate increased in a similar fashion to RBC velocity. At the last hemodilution step, RBC supply rate reached a level 5.2 times the baseline, rather similar to that seen in RBC velocity. RBC velocity and supply rate were positively correlated (r = 0.787, P < 0.001).

To describe the functional dependence of RBC velocity and supply rate on hematocrit, trendlines were fitted by linear regression to data as shown in Fig. 3. For RBC velocity, the best fit was obtained with the logarithmic model: y = −4.134 log(x) + 7.134 (r = 0.834). The measured supply rate data exhibited relatively large variance. Therefore, these data were normalized in each capillary to the mean of all data measured in that capillary. The coefficient of variation calculated from the residual sum of squares after linear regression on hematocrit was 0.71. With normalization, this value was reduced to 0.36. The best fit of normalized RBC supply rate was as follows: y = −2.762 log(x) + 4.955 (r = 0.752).

Lineal cell density, an index of capillary (tube) hematocrit, was normalized in each capillary to the mean of data measured in that capillary and plotted as a function of arterial hematocrit. As seen in Fig. 3, there was no predictable change in cell density during hemodilution; i.e., the mean change in cell density with decreasing arterial hematocrit was zero.

Fig. 2. Effect of isovolemic hemodilution on RBC velocity (A) and mean arterial pressure (MAP; B). In A, each data point represents an average of RBC velocities measured in 4–8 cortical capillaries in the same microscope field. Different symbols designate different experiments. MAP data from the same experiments are identified with corresponding symbols. Data were obtained along the curves from right to left as arterial hematocrit was reduced by stepwise replacement of arterial blood by serum albumin. MAP was supported with intravenous methoxamine.

Fig. 3. Effect of isovolemic hemodilution on RBC velocity (A), supply rate (B), and lineal cell density (C) in cerebrocortical capillaries. RBC supply rate and cell density data were normalized in each capillary to mean of values measured in that capillary. Trendlines are from the best fit of a logarithmic model. Note that RBC velocity and supply rate increase in a similar fashion. Lineal cell density data and regression suggest independence of cerebral capillary hematocrit from systemic arterial hematocrit.
DISCUSSION

The major findings of this study are 1) velocity and supply rate of RBC in cerebral capillaries increases in parallel during isovolemic hemodilution; 2) both variables continue to rise at arterial hematocrits as low as 15%; and 3) lineal cell density, an index of capillary hematocrit, is independent of arterial hematocrit.

A number of former studies (2, 5, 10, 13, 19, 23, 28, 30) confirmed the effect of hemodilution on cerebral blood flow; however, a significant enhancement of RBC perfusion in the cerebral capillary bed has not been previously demonstrated. Cerebral blood flow is increased by active vasodilation (28, 30, 32) and a decrease in blood viscosity (13, 20, 30). The vasodilation is intraparenchymal (28, 32); large cerebral and pial arteries fail to consistently dilate (9, 19, 20, 23) during hemodilution. In addition, hemodilution may facilitate the rate of entry of RBCs into the capillaries by reducing plasma skimming and augmenting the network Fahraeus effect (21). In support of this idea, Rosenblum (25) performed a series of studies altering hematocrit and plasma viscosity by various means in the mouse and found that RBCs and plasma did not have the same path lengths and that the alterations in the degree of plasma skimming influenced the path lengths and velocities of RBC and plasma in different ways. During anemia, RBC velocity in pial vessels increased, whereas plasma velocity in intraparenchymal vessels decreased (23). In the mesenteric and skeletal muscle microcirculations, isovolemic hemodilution altered the distribution of flow among the capillaries such that RBC flow selectively increased in poorly perfused capillaries (21, 29). Hemodilution may also lower capillary flow resistance and enhance RBC flow due to the removal or structural alteration of the endothelial glyocalyx (22).

Consistent with the parallel increase in RBC velocity and supply rate, we found no change in lineal cell density at any level of hemodilution. Lineal cell density is proportional to capillary (tube) hematocrit, and its change reflects the change in capillary hematocrit if capillary diameter remains constant. The hypothesis of constant or increased hematocrit during hemodilution has been known from studies in the peripheral microcirculation (14, 16–18). This view has not been generally held for the cerebral circulation, however. Levin and Ausman (15) found that cerebral cortical hematocrit varied linearly with peripheral hematocrit. Similarly, Todd et al. (28) reported large decreases in cerebral microvascular hematocrit that exceeded the decrease in arterial hematocrit. Several factors may have contributed to the difference between these and our findings. First, we measured cell density in single capillaries, whereas both Levin and Ausman (15) and Todd et al. (28) measured hematocrit in a mixed population of microvessels. The change in hematocrit in arterioles may parallel the change in systemic hematocrit, whereas capillary hematocrit may behave differently, because of the divergence of RBC and plasma flow (24). Second, if capillary diameter increased during hemodilution, then lineal cell density may have overestimated capillary hematocrit. We have no observation to support the idea that capillary diameter increased in our study, but there have been indications of capillary dilation in the brain during hypercapnia (1, 3, 31). Nevertheless, capillary dilation would not affect our conclusions with respect to lineal cell density. Finally, the reported difference could be related to a limitation inherent to the labeled-cell technique. The velocity of fluorescently labeled RBCs cannot be measured during no-flow periods and therefore may overestimate the time-average RBC velocity if capillary flow were intermittent. An intermittence in RBC perfusion of cerebral capillaries has been noted under resting conditions (11, 26, 31). If capillary perfusion became more continuous during hemodilution, then RBC velocity may have increased and lineal cell density may have decreased slightly more than measured in this study.

The finding that RBC supply rate increased considerably during hemodilution has important implications on oxygen transport to cerebral tissue. The concept of optimal hematocrit (5) postulates that oxygen delivery to the brain is maximal at an arterial hematocrit of 30%. However, the consistent increase in RBC supply rate together with maintained arterial P O2 suggest that in our experiments oxygen supply was maintained in the face of decreasing arterial hematocrit to a level as low as 15%. This finding is reinforced by the experiments of Bauer et al. (2), who demonstrated that neuronal function and high-energy phosphates in the brain were preserved at a systemic hematocrit of 6.1%. It is important that in our study arterial blood pressure was maintained to support organ perfusion.

A corollary of these results is that hypoxia could not play a role in hemodilution-induced cerebral hyperemia. In all experiments, arterial P O2 remained above 50 Torr, the threshold for hypoxic cerebral vasodilation (4). Therefore, the vasoregulatory mechanism involved in the hemodilution-induced flow increase was unlikely to be oxygen dependent. We speculate that the principal mechanism of blood flow increase could be the elevation in blood fluidity and shear-dependent cerebral vasodilation.

In summary, the present results show that in the cerebrocortical capillary network both velocity and supply rate of RBC increase and lineal cell density remains unchanged as the arterial hematocrit is reduced by isovolemic hemodilution at constant MAP. The optimal systemic hematocrit is not higher than 15%.

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REFERENCES

HEMODILUTION AND CEREBRAL CAPILLARY FLOW


