Effect of hematocrit variations on cerebral blood flow and basilar artery diameter in vivo

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Muizelaar, J. Paul, Gerrit J. Bouma, Joseph E. Levasseur, and Hermes A. Kontos. Effect of hematocrit variations on cerebral blood flow and basilar artery diameter in vivo. Am. J. Physiol. 262 (Heart Circ. Physiol. 31): H949–H954, 1992.—Despite observations that pial arterioles constrict with decreased blood viscosity or hemodilution, several investigators have found an inverse relationship between cerebral blood flow (CBF) and hematocrit (Hct) under physiological conditions. To investigate whether this is due to a dilation of the more proximal large cerebral arteries, in vivo responses of CBF and basilar artery to hemodilution and hemoconcentration were studied in 21 anesthetized normal cats, using a closed clival window model. An inverse correlation between Hct and CBF was found, but CBF responses were smaller than previously reported data suggest. Varying Hct between 60 and 120% of baseline caused CBF to vary between 140 and 90%, approximately. Moderate hemodilution was associated with a significant decrease (−4.1%) in basilar artery diameter (P < 0.05), but other Hct manipulations had no consistent effect on basilar artery diameter. It is concluded that dilation of large cerebral arteries cannot account for the decreased cerebrovascular resistance following hemodilution but that a disproportionate reduction of in vivo viscosity must be responsible. Pial arteriolar constriction after hemodilution therefore probably reflects a normal autoregulatory adjustment of vasomotor tone to altered blood rheology, whereas changes in large artery caliber may serve to modulate microvascular pressure.

On the assumption that recruitment (opening of previously closed parts of the microcirculation) was not responsible for the increase in CBF, Hudak et al. calculated the viscosity changes in the microcirculation secondary to the Hct changes and concluded that the net reduction in arteriolar and capillary vascular resistance (effect of decreased viscosity minus effect of vasoconstriction) was not sufficient to explain the increased CBF. Then they generated a new hypothesis, namely that the arteriolar constriction is counteracted by presumed vasodilation of the larger, more proximal cerebral vessels, thus explaining the net reduction in cerebrovascular resistance (CVR). If found to be true, this theory would have two important implications. First, it would lend further credence to the calculations by Hudak et al. of viscosity in the microcirculation. Second, it would assign a new regulatory role to the proximal part of the cerebral circulation (although its mechanism would remain unexplained as yet).

To investigate the hypothesis that hemodilution causes dilatation of large cerebral arteries, we have measured basilar artery diameter along with CBF during isovolemic manipulations of Hct in cats. The inclusion of experimental isovolemic hemoconcentration into the protocol would allow us, for the first time, to investigate its effect on CBF. Our secondary hypothesis was that hemoconcentration would attenuate CBF and cause constriction of the basilar artery.

Materials and Methods

Animal preparation. Twenty-one adult cats of mixed sex and breed, each weighing 3.0–4.0 kg, were used in this study. The protocol was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The animals were anesthetized with 30 mg/kg iv pentobarbital sodium. Polyvinyl chloride catheters were inserted into a femoral artery and vein. Arterial blood pressure was monitored via a Statham pressure transducer. Additional intravenous pentobarbital was given as needed to maintain constant blood pressure and miotic pupils. Tracheostomy was performed through a midline cervical incision. To facilitate later exposure of the clivus while maintaining an adequate airway, the tracheostomy was placed low in the neck. Ventilation was regulated with a Harvard animal respirator, and skeletal muscle paralysis was induced with 5 mg/kg gallamine triethiodide. End-tidal Pco2 was monitored continuously with a Hewlett-Packard 47210A capnometer. The respiratory rate and volume were adjusted so as to maintain expiratory Pco2 at 30 mmHg. Arterial blood gases were obtained at regular intervals using a Corning 158 pH/blood gas analyzer. Rectal temperature was maintained with an electric thermometer and kept at 37–38°C using an electric heating pad. The head was placed in a stereotactic frame, a left parietal craniectomy was carried out, and after opening of the...
H950

EFFECT OF HEMATOCRIT ON CBF AND BASILAR ARTERY

dura mater, a Peltier Stack thermal diffusion CBF probe (Flowtronics model 6000-01) as described by Carter et al. (5) was placed on the cortex for continuous measurement of CBF. The skull opening and probe were covered with bone wax and dental acrylic in a watertight fashion. With the head still fixed in the stereotactic frame, the animal was then turned in the supine position. The basilar artery was exposed by a translcal approach, and after opening of the dura mater, a Plexiglas window was installed into the clivus (see Ref. 2 for details). This preparation allows direct observation of the artery and repeated measurements of vessel diameter, without introducing artifacts due to loss of CO₂ from the cerebrospinal fluid (CSF) via the exposed surface (28). Vessel diameter was measured using a Vickers image splitter (Device AEI), attached to a Leitz microscope with a ×3.6 dry objective. Calibration of the image splitter for use with the ×3.8 objective had been carried out before on a micrometer scale fixed to a glass plate. Calibration was reproducible and linear in the range between 0 and 800 μm.

The diameter of the basilar artery was on each occasion measured at three distinguished separate points; the arithmetic mean of these three measurements was taken as the vessel diameter at that time.

Experimental protocol. Before the measurements were started, 30–60 min were allowed for stabilization of vessel diameter and other physiological parameters. Baseline measurements of mean arterial blood pressure (MABP), blood gases, CBF, and basilar artery were obtained. To assess cerebrovascular reactivity, the animals were hyperventilated to an endtidal Pco₂ of 18 mmHg and all measurements repeated. Next, normocapnia was reestablished and measurements were repeated once more. If an animal did not show a normal CBF response to hypocapnia (at least 1.5% change in CBF per Torr CO₂), it was considered to have abnormal vasoreactivity and thus was excluded from the studies. (In this series, however, all animals had a normal CBF response to hypocapnia.)

The animals were each assigned to one of three groups. Group A consisted of 13 cats that, on completion of the baseline physiological measurements, underwent two successive isovolumic hemodilutions followed by an isovolumic hemococoncentration. In group B (5 cats), the order of Hct manipulations was reversed: after the baseline measurements, isovolumic hemodilution preceded two consecutive isovolumic hemodilutions. Finally, group C consisted of 3 cats who served as controls for experimental groups A and B.

Measurements in control group C indicated that the preparation itself did not influence any physiological parameters or basilar artery diameter (Table 3).

DISCUSSION

Although an inverse correlation between Hct and CBF has been well demonstrated in patients with cerebral circulatory disorders (12, 33, 35) and in animal models of cerebral ischemia (30, 36), data on the physiological relationship between these two parameters are less consistent (3, 9, 13, 14, 17, 19, 23, 29, 32). Grossly, alterations in Hct may affect CBF by two mechanisms: altered blood viscosity with subsequent reduced vascular resistance and altered O₂ content leading to metabolically mediated vasodilation or constriction to maintain O₂ transport to the brain. Whereas O₂ content has been shown to greatly influence CBF under normal physiological conditions (4), viscosity per se appears to have little effect on CBF (3), presumably due to autoregulatory adaptations of microvascular diameter (25, 27).

Microcirculatory vessel diameter changes in response to blood viscosity alterations had previously been demonstrated by Muizelaar et al. (26), but in that study CBF was not measured. Recently, however, Hudak et al. (18) have confirmed that hemodilution causes constriction of pial arteries and arterioles, which appeared to be maximal at an Hct of ~70% of baseline value, accompanied by an increase in CBF. The authors concluded that a decrease in vascular resistance elsewhere in the cerebro-

RESULTS

Tables 1 and 2 show means ± SD of the physiological parameters at baseline and after each Hct manipulation for experimental groups A and B, respectively. These manipulations significantly altered Hct in the animals of both groups while MABP and blood gases remained stable.

Baseline CBF (CBF₀) varied considerably and ranged between 29.7 and 70.4 ml·100 g⁻¹·min⁻¹. To correct for the baseline differences, parameters are expressed as fractions of their baseline values. In all animals, CBF varied inversely with Hct (Fig. 1), and the regression of CBF/CBF₀ to Hct₀/Hct was significant (r = 0.64, P < 0.001; Hct₀, baseline hematocrit). The maximum amount that CBF would increase with hemodilution was found to be 65.5% of baseline.

The baseline diameter of the basilar artery in all animals varied from 324 to 646 μm (mean, 511 ± 81 μm). Variations in Hct did not have a consistent effect on the diameter of the basilar artery, regardless of the direction or the order of the various manipulations (Fig. 2). Changes in basilar artery diameter ranged between −25.7 (vasoconstriction) and +9.7% (vasodilation). A weak (4.4 ± 5.0%) but significant constriction was found with moderate hemodilution in group A (P < 0.05). Changes from baseline in basilar artery diameter with further hemodilution or hemoconcentration were not significant.

Measurements in control group C indicated that the preparation itself did not influence any physiological parameters or basilar artery diameter (Table 3).
Table 1. Physiological parameters in group A: two successive hemodilutions followed by hemoconcentration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>First Hemodilution</th>
<th>Second Hemodilution</th>
<th>Hemoconcentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct, %</td>
<td>28.0±3.9</td>
<td>21.4±2.8*</td>
<td>16.9±3.1*</td>
<td>22.5±2.8*</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>87.8±5.9</td>
<td>91.6±8.7</td>
<td>89.8±6.4</td>
<td>93.0±7.5</td>
</tr>
<tr>
<td>Pao₂, mmHg</td>
<td>100±25.6</td>
<td>98.1±24.6</td>
<td>96.8±24.8</td>
<td>101.2±22.0</td>
</tr>
<tr>
<td>Paco₂, mmHg</td>
<td>31.8±2.1</td>
<td>32.2±3.3</td>
<td>32.2±2.3</td>
<td>32.2±2.9</td>
</tr>
<tr>
<td>CBF, ml/100 g⁻¹.min⁻¹</td>
<td>46.5±11.7</td>
<td>52.6±14.8*</td>
<td>60.6±16.4*</td>
<td>52.3±10.5*</td>
</tr>
<tr>
<td>BA diameter, μm</td>
<td>522±76</td>
<td>498±77†</td>
<td>507±76</td>
<td>481±89</td>
</tr>
<tr>
<td>BA diameter, % of baseline</td>
<td>95.6±5.0†</td>
<td>99.1±6.1</td>
<td>95.0±9.1</td>
<td></td>
</tr>
<tr>
<td>Vascular hindrance, % of baseline</td>
<td>122.9±25.6†</td>
<td>108.0±28.4</td>
<td>137.4±68.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 13 cats. Measurements were obtained before and after manipulations of hematocrit (Hct). MABP, mean arterial blood pressure; Pao₂, arterial oxygen pressure; Paco₂, arterial carbon dioxide pressure; BA, basilar artery. Significantly different from baseline (paired t test): * P < 0.01; † P < 0.05.

Table 2. Physiological parameters in group B: hemoconcentration (isovolumic red cell infusion) followed by two successive hemodilutions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hemoconcentration</th>
<th>First Hemodilution</th>
<th>Second Hemodilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct, %</td>
<td>30.6±2.8</td>
<td>36.8±2.5*</td>
<td>24.8±3.0*</td>
<td>20.0±2.4*</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>93.0±10.4</td>
<td>98.2±9.2</td>
<td>92.8±6.4</td>
<td>88.3±2.5</td>
</tr>
<tr>
<td>Pao₂, mmHg</td>
<td>92.8±11.1</td>
<td>92.7±14.2</td>
<td>84.6±7.2</td>
<td>90.5±15.3</td>
</tr>
<tr>
<td>Paco₂, mmHg</td>
<td>33.0±3.5</td>
<td>33.0±2.5</td>
<td>32.6±3.1</td>
<td>33.3±2.9</td>
</tr>
<tr>
<td>CBF, ml/100 g⁻¹.min⁻¹</td>
<td>42.2±4.2</td>
<td>38.0±3.5†</td>
<td>47.3±5.1*</td>
<td>52.9±8.8*</td>
</tr>
<tr>
<td>BA diameter, μm</td>
<td>471±89</td>
<td>446±89</td>
<td>439±123</td>
<td>446±133</td>
</tr>
<tr>
<td>BA diameter, % of baseline</td>
<td>95.1±7.5</td>
<td>91.7±12.2</td>
<td>91.6±8.2</td>
<td></td>
</tr>
<tr>
<td>Vascular hindrance, % of baseline</td>
<td>171.2±96.5</td>
<td>155.3±62.3</td>
<td>130.3±39.7</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 cats. Measurements were obtained before and after manipulations of hematocrit. Significantly different from baseline: * P < 0.01; † P < 0.05.

Fig. 1. Cerebral blood flow (CBF), expressed as percentage of baseline value vs. ratio of hematocrit at time of measurement to baseline hematocrit (Hct/Hctₐ). Regression equation is shown in graph.

Fig. 2. Percentage change in basilar artery diameter vs. Hct/Hctₐ.
dilution caused a modest increase in CBF but did not dilate the basilar artery. Instead, with a moderate level of hemodilution, a mild constriction of this vessel occurred. It is unlikely that the weight of the thermal diffusion flow probe (1.5 g) prevented cortical blood flow from increasing in response to the experimental manipulations, because it has been demonstrated that application of weights up to 5 g on the probe has no effect on the recording (6). These findings would indicate that the decrease in CVR following hemodilution cannot be attributed to dilation of large cerebral arteries. Consequently, the overall decrease of vascular resistance after hemodilution must be the result of a disproportionate fall in blood viscosity (if one assumes that the total number of perfused vessels does not increase), which is not completely compensated by microvascular constriction. We speculate that this reflects the net result of the balance between adjustments of vasomotor tone and decreased viscosity so as to maintain constant O2 transport to the brain. Apparently, when Hct is lowered to 70–75% of the baseline value, viscosity effects are dominating as vasoconstriction occurs despite lower arterial O2 content, whereas with more profound hemodilution the effect of decreased O2 transport to the brain overrides, leading to diminished vasoconstriction or even vasodilation.

A few methodological issues should be considered, however. First, blood flow was measured in an area not primarily supplied by the basilar artery. This should not seriously affect the analyses, however, as only relative changes in CBF were considered, and it seems unlikely that hemodilution would disproportionately affect regional flow in various areas of the brain. Also, the diameter of other large cerebral arteries was not measured, and thus vasodilation of these vessels cannot be completely excluded. However, in cats the bulk of supratentorial blood flow is supplied via the rete mirabile, which can hardly be regarded as a large artery. Although the role of the rete in the regulation of CBF in cats is not completely known, there is no reason to suspect that hemodilution would specifically cause vasodilation of the rete mirabile and constriction of all other cerebral vessels. One might argue that the window preparation itself might impair vascular reactivity of the basilar artery, but we found maximum responses of basilar artery diameter grossly ranging between +10 and −25%, which is in good agreement with other data (11). Earlier, we had found that CO2 reactivity of the basilar artery was preserved with this preparation (2). Finally, it should be noted that Hctb in our animals was somewhat lower than what would be considered normal (29 instead of 32%), which is possibly due to the sometimes lengthy surgical preparation (2). This may to some extent have blunted the viscosity effects of the Hct manipulations because of higher shear rates in the microcirculation. However, this should have attenuated the vasoconstrictor responses rather than enhanced them and does not affect our main conclusion, that hemodilution does not dilate the basilar artery.

Due to the complex interaction between cerebrovascular topography and viscosity factors, it is difficult to separate effects of viscosity and vascular diameter on net vascular resistance to flow. Calculations of vascular hindrance (i.e., the portion of vascular resistance that is not attributed to viscosity) from measurements of flow resistance (pressure/flow ratio) and viscosity, as applied by some workers (9, 18), are valid only under the assumption that determinations of in vitro viscosity can be extrapolated to the microcirculation in vivo. Based on such calculations, Fan et al. (9) found a 27% increase in total vascular hindrance in the brain with hemodilution to ~50% of the baseline value, which corresponds with a 6% decrease in vascular diameter based on Poiseuille’s law. This theoretically calculated vasoconstriction is considerably less than has been found in actual measurements of vessel diameter (18, 26). It is therefore likely that in vivo viscosity changes with alterations of Hct are much larger than in vitro measurements would suggest. This can be demonstrated by quantitative analysis of our data and those of Hudak et al. (18). In the present study, a 25% decline in Hct, from 28 to 21%, was associated with an ~20% rise in CBF. At the same time, basilar artery diameter narrowed by 4.4%. Thus, according to Poiseuille’s law, vascular hindrance in the basilar artery increased by a factor of [1/(1 − 0.044)]^4 = 1.20. Similarly, Hudak et al. (18) found posthemodilution hindrance in the large and small pial arterioles to be increased by factors of 1.67 and 3.33, respectively. Assuming that vascular hindrance in the capillaries and postcapillary venules did not change significantly, we can now roughly estimate the net increase in cerebrovascular resistance after moderate hemodilution. Under normal circumstances, large proximal cerebral arteries (>300 μm) account for ~40% of total CVR (14, 16), intraparenchymal arteries and large pial arterioles (>100 μm) for 30%, small pial arterioles for 10%, and capillaries and veins for the remaining 20%. If the calculated increases in vascular hindrance in each segment are implemented in this ratio, it follows that total posthemodilution cerebrovascular hindrance increased by a factor of 1.51. Because CBF increased by a factor of 1.2, net in vivo viscosity must have decreased by ~40%. It is obvious that this decrease is much larger than the ~15% decline that has been found with in vitro measurements of viscosity in cats, with Hct reduction from 28 to 21% (22).

Several mechanisms may be responsible for a disproportionate change in in vivo blood viscosity with altered Hct. First, hemodilution causes increased flow velocity and subsequently higher shear rates in the microcirculation (31), this progressively decreases viscosity, espe-
cially at a moderate level of hemodilution, as with very low Hct the relationship between shear rate and blood viscosity disappears (22, 34). Second, decreased erythrocyte aggregation resulting from lower Hct and higher shear rates progressively decreases viscosity in the microcirculation (7), and this effect is not measured in vitro. Third, vasoconstriction associated with hemodilution may enhance the Fahraeus-Lindqvist effect (8).

Recent data suggest that large cerebral arteries play a role in regulating microvascular pressure rather than CBF per se (1, 10). When viscosity is lowered, net vascular resistance in the large cerebral arteries decreases, which results in a smaller pressure gradient over these vessels and a subsequent increase in microvascular pressure. This may lead to a compensatory constriction of the larger arteries, although the precise mechanism by which this response is mediated remains to be disclosed. The present observation that moderate hemodilution is associated with a weak but significant constriction of the basilar artery supports this hypothesis.

We conclude that there is no proof for the contention that changes of CBF in response to Hct variations reflect more than a physiological regulation of O2 transport to the brain. We hold that under these altered rheologic circumstances autoregulatory adjustments in vasomotor tone will tune blood flow to metabolism. Consequently, vasoconstriction will occur whenever in vivo viscosity decreases relatively more than O2 delivery to the brain, but when O2 transport is further decreased by subsequent hemodilutions, vasoconstriction will diminish or even vasodilatation may occur. In this context, changes in large artery diameter may serve to regulate microvascular pressure, but this can only be confirmed by direct measurements of microvascular pressure.

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