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Lack of Hypercapnic Increase in Cerebral Blood Flow at High Blood Viscosity in Conscious Blood-exchanged Rats

Christian Lenz, M.D., Annette Rebel, M.D.,* Enrico Bucci, Ph.D.,† Klaus van Ackern, M.D.,‡ Wolfgang Kuschinsky, M.D.,§ Klaus F. Waschke, M.D.‖*

Background: **The hypothesis of a compensatory dilation of cerebral vessels to maintain cerebral blood flow at a high blood viscosity was tested during hypercapnia in the study after replacement of blood by hemoglobin solutions of defined viscosities. If compensatory vasodilation exists at normocapnia at a high blood viscosity, vasodilatory mechanisms may be exhausted when hypercapnia is added, resulting in a lack of increase in cerebral blood flow at hypercapnia.**

Methods: **In conscious rats, blood was replaced by ultrapurified cross-linked hemoglobin solutions that had defined and shear rate–independent low or high viscosities (low- and high-viscosity groups). Blood viscosity differed threefold between both groups (1.2 *vs.* 3.6 mP · s). Thereafter, rats inhaled either a normal or an increased concentration of carbon dioxide in air. Cerebral blood flow was determined by the iodo[14C]antipyrine method.**

Results: **During normocapnia, global and local cerebral blood flows did not differ between both groups. With increasing degrees of hypercapnia, global and local cerebral blood flows were gradually elevated in the low-viscosity group (2.8 ml · $mmHg^{-1}$ CO_2 · 100 g^{-1} · min^{-1}), whereas they remained unchanged in the high-viscosity group.**

Conclusions: **Changes in blood viscosity do not result in changes of cerebral blood flow as long as cerebral vessels can compensate for these changes by vasodilation or vasoconstriction. However, such vascular compensatory adjustments may be exhausted in their response to further pathophysiologic conditions in blood vessels that have already been dilated or constricted as a result of changes in blood viscosity.**

THE impact of blood viscosity on cerebral blood flow is still under discussion. The following hypotheses have been raised during previous investigations: (1) Changes in blood viscosity induce reciprocal changes in cerebral blood flow[1–3] and (2) changes in blood viscosity do not induce changes in cerebral blood flow. Compensatory responses of the brain vessels keep the cerebral blood flow constant. The ultimate driving force for cerebral blood flow is the maintenance of oxygen delivery to the brain.[4–9]

However, in most of these previous studies, blood viscosity was varied by changes in the hematocrit.[1,3,4,6,7] This approach does not allow a definite conclusion concerning whether viscosity or oxygen delivery determines cerebral blood flow because a lower blood viscosity at a lower hematocrit is inevitably associated with a lower arterial oxygen content. In addition, the non-newtonian properties of blood and the unknown shear rates that exist in the circulation make it impossible to extrapolate from viscosimetric data of blood obtained *in vitro* to the viscosity of blood in the cerebral microcirculation *in vivo*.[10] Both difficulties can be circumvented when blood is exchanged by an ultrapurified cross-linked hemoglobin solution. After such an exchange, defined and constant blood viscosities can be obtained in the circulation, including the cerebral microcirculation. In addition, in contrast to fluids used for hemodilution, hemoglobin solutions contain oxygen carriers that give the opportunity to maintain an identical oxygen content of the blood in experimental groups of different blood viscosities.

Using an ultrapurified, polymerized hemoglobin solution as a blood substitute in a previous study,[11] our group could demonstrate that replacement of blood by hemoglobin solutions of fourfold varying viscosities did not result in differences in cerebral blood flow in normocapnic rats. These results can be explained by the existence of vasodilating mechanisms in the cerebral vessels that allow compensation for differences in blood viscosity resulting in a maintenance of cerebral blood flow. The hypothesis of a compensatory vasodilation that occurs during normocapnia at a high blood viscosity can be tested during hypercapnia. In this case, the dilating mechanisms may be exhausted to compensate for the high blood viscosity, resulting in a reduced or abolished increase in hypercapnic cerebral blood flow at a high blood viscosity. To obtain defined and shear rate–independent blood viscosities, blood was exchanged for ultrapurified cross-linked hemoglobin solutions. One group of rats was given a high-viscosity solution and the other a low-viscosity solution by exchange transfusion. The animals of both groups were exposed to different carbon dioxide concentrations in the inspiratory air, and

* Research Fellow, ‡ Professor of Anesthesiology and Chair, ‖ Research Coordinator, Department of Anesthesiology, Faculty of Clinical Medicine Mannheim, University of Heidelberg. † Professor of Biochemistry, Department of Biological Chemistry, University of Maryland at Baltimore, Baltimore, Maryland. § Professor of Physiology and Chair, Department of Physiology, University of Heidelberg, Heidelberg, Germany.

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Address reprint requests to Dr. Lenz: Institut für Anästhesiologie und Operative Intensivmedizin, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, Theodor Kutzer Ufer 1-3, D-68167 Mannheim, Germany. Address electronic mail to: jr1@ix.urz.uni-heidelberg.de. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

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cerebral blood flow was determined by the iodo[^{14}C]antipyrine method.

Materials and Methods

After approval by the institutional animal care committee (Regierungspräsidium Karlsruhe, Karlsruhe, Germany) the experiments were performed on 20 male Sprague-Dawley rats (Charles River Deutschland, Sulzfeld, Germany) weighing 291 ± 27 g (mean ± SD). The animals were kept under temperature-controlled environmental conditions on a 14:10 light:dark cycle and were fed a standard diet (Altromin 1324; Altromin, Lage, Germany) with free access to food and potable water until the start of the experiments.

The rats were placed in a small box and anesthetized by inhalation of halothane, 4% (Fluothane; Zeneca, Plankstadt, Germany); N_2O, 70%; and oxygen, remainder. Anesthesia for surgery was maintained by halothane, 1.0–1.5%, administered through a nose cone. Body temperature was held at 37°C by a temperature-controlled heating pad. Polyethylene catheters (PE-50; Labokion, Sinsheim, Germany) were inserted into the right-side femoral artery and vein. All wound sites were infiltrated with bupivacaine. The arterial catheter was connected to a pressure transducer for continuous blood pressure monitoring, and the venous catheter was used for drug and fluid administration. After surgery, the animals were placed in a rat restrainer (Braintree Scientific, Braintree MA) and allowed to recover for a minimum of 3 h before the experiments were continued.

Blood gas levels were measured using an automated blood gas analyzer (AVL Gas Check Model 939; AVL, Graz, Austria). Hemoglobin content, oxygen saturation, total oxygen content, and methemoglobin content were measured using a hemoximeter (Model OSM-3; Radiometer, Copenhagen, Denmark). The hematocrit was determined by capillary tube centrifugation (Haematokrit Model 2011; Hettich, Tuttlingen, Germany). Plasma concentration of sodium and potassium were measured by flame photometry (Model 480 Flame photometer; Corning, Halstead, United Kingdom). Plasma chloride concentration was determined with a chloride meter (Chloridmeter Model 6610; Eppendorf, Hamburg, Germany). Plasma glucose concentration was measured by a polarographic method (Glucose Analyzer Model 2; Beckmann, München, Germany). Plasma osmolality was measured by a vapor pressure osmometer (Model 5500 vapor pressure osmometer; Wescor Inc., Logan, UT). Oncotic pressures were determined by use of a colloid osmometer with a 20-kD membrane (Onkometer BMT 921; Dr. Karl Thomae GmbH, Biberach, Germany). Blood viscosity was measured by a rotating cone-plate viscometer (Model DV-2; Wells-Brookfield, Middleboro, MA) with a 0.8° cone spindle (model No. CP 40). Immediately before measurement of cerebral blood flow, 500 μl blood was drawn from each animal into heparinized Eppendorf tubes and replaced by the appropriate hemoglobin solution. Thereafter, viscosity of this blood was measured at five different shear rates (22.5, 45, 90, 225, and 450 s^{-1}), starting with the lowest shear rate. The value of blood viscosity was read when it had stabilized on the display. After the highest shear rate had been reached, the measurements were repeated in each sample in the reverse order. The mean of the two measurements at each shear rate was used for further calculations.

The hemoglobin solution used in the present study had been prepared from outdated human blood units by cross-linking the β-82-lysines by a sebacyl bridge.[12] *In vitro* this cross-linked hemoglobin solution (cHb) has a partial pressure of oxygen at which oxygen saturation of hemoglobin amounts to 50% (P_{50}) of 32 mmHg and a cooperativity value of n = 2.0.[12] To increase its viscosity, polyvinylpyrrolidone (PVP), 2% (molecular weight, 1,100,000) (polyvinylpyrrolidone 90 Serva; Boehringer Ingelheim Bioproducts Partnership, Heidelberg, Germany), was added to a part of the hemoglobin solution (cHb-PVP).

Preliminary experiments in a previous study[11] had shown that the blood viscosity obtained by blood exchange with a hemoglobin solution with a viscosity that had been increased by addition of PVP, 2%, was the maximum increase in blood viscosity the animals could tolerate. A further increase induced circulatory failure and death. Therefore, in the present investigation an identical concentration of PVP was chosen to increase the viscosity of the hemoglobin solution used in the high-viscosity group.

Solutions of PVP have been used as plasma expanders since 1942.[13] The only pharmacodynamic actions of polyvinylpyrrolidone on blood vessels and body tissues observed since 1942 are its uptake by the reticuloendothelial system and subsequent granuloma formation after prolonged administration.[14]

Because of restricted availability of the blood substitute, the rats were first hemodiluted by albumin, 5% (human albumin, 5%; Immuno, Heidelberg, Germany), to a hematocrit of approximately 20%. Then blood was exchanged for cHb (table 1) in 10 rats (low-viscosity group), whereas in another 10 rats, blood was exchanged for cHb-PVP (high-viscosity group) (table 1). CHb-PVP was prepared freshly for each experiment, and both solutions were warmed to 37°C before measurement of their characteristics and application for hemodilution. Blood losses from arterial blood sampling were replaced either by albumin before blood exchange and by hemoglobin solution after blood exchange.

Thirty minutes after blood exchange the rats in both groups were exposed to carbon dioxide in the inspired air. To induce a different arterial carbon dioxide tension (Pa_{CO_2}) in the range of 35–100 mmHg in each animal, an

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Table 1. Characteristics of Hemoglobin Solutions

	cHb	cHb-PVP
Hemoglobin concentration (g/dl)	8.2 ± 0.9	8.2 ± 0.9
Methemoglobin (% of hemoglobin concentration)	19.9 ± 8.9	24.3 ± 6.3
pH	7.0 ± 0.2	7.0 ± 0.2
Oncotic pressure (mmHg)	26 ± 5	47 ± 8*
Osmolality (mOsm/kg)	286 ± 9	287 ± 14
Sodium concentration (mM)	149 ± 1	145 ± 1*
Potassium concentration (mM)	4.14 ± 0.02	4.05 ± 0.05*
Chloride concentration (mM)	118 ± 6	115 ± 4
Viscosity (mPa · s)	1.5 ± 0.3	5.8 ± 0.7*

Values are mean ± SD. Viscosity values are based on shear rates of 22.5–450 mPa · s.

* $P < 0.05$ *versus* cross-linked hemoglobin solution (cHb).

PVP = polyvinylpyrrolidone.

individual concentration of carbon dioxide in the inspired air was selected for each animal in both groups, including one animal in each group exposed to CO_2, 0%. For preparing various concentrations of carbon dioxide in the inspired air, pressurized carbon dioxide was connected to the oxygen rotameter and pressurized air to the NO_2 rotameter of a Sulla anesthesia machine (Dräger, Lübeck, Germany). A hose for conducting the gas mixture was fixed to the nose cone of the rat tunel immediately before the beginning of the gas exposure, and the gap between nose cone and the guillotine used to kill the animal was occluded by adhesives. Preliminary experiments had shown that by changing the rotameters flow rate, this setting allowed for induction of predictable ranges of Pa_{CO_2} between 40 and 100 mmHg in spontaneously breathing rats, although it was not possible to trigger Pa_{CO_2} to exact values. Five minutes after the beginning of exposure to carbon dioxide, a value for Pa_{CO_2} was obtained and the measurement of cerebral blood flow was prepared, which lasted approximately 5 min. Immediately before measurement of cerebral blood flow, a second value of Pa_{CO_2} was obtained for proof of stabilization of Pa_{CO_2} and for use in further calculations.

Local cerebral blood flow (LCBF) was measured by infusing 100 μCi/kg body weight 4-iodo-N-methyl-[^{14}C]antipyrine (specific activity, 55 μCi/mM; American Radiolabeled Chemicals, St. Louis, MO) dissolved in 1 ml saline continuously at a progressively increasing infusion rate for 1 min through the femoral venous catheter. The progressively increasing infusion rate, a modification of the method described previously,[15] was chosen to minimize equilibration of rapidly perfused tissues with arterial blood during the period of measurement. During the 1-min infusion period, 14–20 timed blood samples were collected in drops from the free-flowing arterial catheter directly onto filter paper disks (1.3 cm in diameter) that previously had been placed in small plastic beakers and weighed. The samples were weighed and radioactivity estimated using a liquid scintillation counter (Tri-Carb 4000 series; Canberra Packard, Frankfurt, Germany) after extraction of the radioactive compound with ethanol. After the 1-min infusion and sampling period, the animal was decapitated; the brain was removed as quickly as possible and frozen in 2-methylbutane chilled to −40 to −50°C. The frozen brains were coated by chilled embedding medium (Lipshaw, Detroit, MI), stored at −80°C in plastic bags, sectioned into 20-μm sections at −20°C in a cryostat, and autoradiographed along with precalibrated [^{14}C]methyl methacrylate standards. Autoradiographic images were converted to digitized optical density images by an image processing system (MCID; Imaging Research, St. Catharines, Ontario, Canada). Tissue optical densities were converted to [^{14}C] concentration by comparison with the precalibrated standards. LCBF was calculated from the local concentrations of [^{14}C] and the time course of the blood 4-iodo-N-methyl-[^{14}C]antipyrine concentrations, including corrections for the lag and washout in the arterial catheter.[15] The washout correction rate constant was 100/min. A brain–blood partition coefficient of 1.1 for iodo[^{14}C]antipyrine was used as previously determined for hemoglobin solution and brain tissue in our strain of rats.[16]

For measurements of separate brain structures an ellipsoid cursor was used and adjusted to the size of the individual region. For measurement of mean cerebral blood flow, coronal sections were analyzed as a whole at distances of 200 μm and the values were summarized to obtain the area-weighted means of all measured sections.

Statistical Analysis

After testing for normal distribution of the data, statistical differences between the experimental groups were evaluated by analysis of variance and paired and unpaired *t* tests with Bonferroni correction for multiple comparisons. The overall relation between Pa_{CO_2} and mean cerebral blood flow was assessed by the least-squares fit of the data to $y = ax + b$ where x is the Pa_{CO_2} and y is mean cerebral blood flow. The significance of the coefficients of correlation obtained against zero was tested according to R. A. Fisher. Confidence intervals for the coefficients of correlation were derived by David.[17]

Results

Physiologic Parameters

Table 2 shows the physiologic parameters of both experimental groups before the initial hemodilution and after blood exchange. Most systemic parameters were either unchanged or moderately altered after blood exchange. In contrast, hematocrit, hemoglobin concentration, and arterial oxygen content were significantly reduced, whereas methemoglobin content and oncotic pressure were significantly increased after blood ex-

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Table 2. Physiologic Variables before and after Blood Exchange

	Baseline (After Recovery from Anesthesia)		After End of Blood Exchange	
	cHb	cHb-PVP	cHb	cHb-PVP
Mean arterial blood pressure (mmHg)	120 ± 19	109 ± 17	105 ± 17*	100 ± 21
Heart rate (min^{-1})	402 ± 39	445 ± 44†	440 ± 73	406 ± 64
Hematocrit (%)	43.3 ± 1.8	44.4 ± 2.4	2.4 ± 0.4*	2.1 ± 0.4*
Hemoglobin concentration (g/dl)	14.6 ± 0.7	14.7 ± 0.7	6.8 ± 0.7*	5.9 ± 0.5*†
Methemoglobin (% of hemoglobin concentration)	0.7 ± 0.2	0.7 ± 0.2	15.5 ± 6.6*	19.7 ± 4.3*†
Arterial pH	7.41 ± 0.04	7.43 ± 0.03	7.38 ± 0.03*	7.36 ± 0.06*
Pa_{O_2} (mmHg)	94 ± 6	96 ± 7	105 ± 8*	112 ± 8*†
Pa_{CO_2} (mmHg)	42 ± 5	41 ± 4	34 ± 4*	31 ± 5*
Ca_{O_2} (ml/dl)	18.8 ± 1.1	19.1 ± 1.1	7.2 ± 0.4*	5.8 ± 0.3*†
Plasma glucose concentration (mg/dl)	158 ± 12	149 ± 22	177 ± 36	152 ± 50.7
Plasma sodium concentration (mM)	144 ± 1	146 ± 1†	140 ± 3*	140 ± 3.4*
Plasma potassium concentration (mM)	3.9 ± 0.4	3.9 ± 0.3	3.5 ± 0.6*	4.0 ± 0.9
Plasma chloride concentration (mM)	100 ± 6	100 ± 4	103 ± 6	101 ± 2.9
Plasma osmolality (mOsm/kg)	285 ± 10	279 ± 8	286 ± 11	287 ± 4.8*
Plasma oncotic pressure (mmHg)	19 ± 2	18 ± 2	26 ± 3*	30 ± 11*

Values are mean ± SD.

* $P < 0.05$ *versus* baseline. † $P < 0.05$ *versus* cross-linked hemoglobin solution (cHb).

PVP = polyvinylpyrrolidone; Pa_{O_2}, arterial oxygen tension; Pa_{CO_2} = arterial carbon dioxide tension; Ca_{O_2} = arterial oxygen content.

change. Comparison between the low-viscosity group and the high-viscosity group also showed no difference in most systemic parameters, although hemoglobin and arterial oxygen content in the high-viscosity group was slightly lower after blood exchange and methemoglobin content and arterial oxygen tension (Pa_{O_2}) were slightly higher compared with the low-viscosity group.

Viscosimetric Data

After near-total blood replacement (hematocrit < 3%) whole-blood viscosity was found to be shear rate–independent in both experimental groups. Blood exchange by cHb-PVP in the high-viscosity group resulted in a threefold higher blood viscosity compared with blood exchange by cHb in the low-viscosity group (3.6 ± 0.1 *vs.* 1.2 ± 0.1 mP · s [mean ± SD of all blood viscosity measurements performed at the given five different shear rates after blood exchange]).

Mean Cerebral Blood Flow

Figure 1 shows the results of the measurement of mean cerebral blood flow at different values of Pa_{CO_2} in the two rat groups (cHb *vs.* cHb-PVP). During normocapnia, mean cerebral blood flow was not different in the two groups. With increasing degrees of hypercapnia, carbon dioxide reactivity was preserved in the low-viscosity group: Mean cerebral blood flow was increased with increased Pa_{CO_2} resulting in a slope of the regression line of 2.8 (r = 0.85, $P < 0.01$ different from zero; 95% confidence interval [CI]: 0.48 < r < 0.95). In contrast, the high-viscosity group showed no correlation between mean cerebral blood flow and Pa_{CO_2} (r = 0.29 not different from zero [$P < 0.05$]; 95% CI: −0.39 < r < 0.76). The slope of the regression line (0.7) was significantly lower in the high-viscosity group than in the low-viscosity group ($P < 0.05$).

Local Cerebral Blood Flow

The differences in cerebral blood flow between both groups observed for the whole brain were also reflected on a local level in the 36 brain structures investigated. In fig. 2 the results of the local measurements of cerebral blood flow have been pooled into four groups of rats according to the Pa_{CO_2} (Pa_{CO_2} < 40, 40–60, and 60–80 mmHg and > 80 mm Hg). However, because LCBF values obtained from multiple brain structures in a single animal are not independent from each other and because of the small number of observations in each group, it was not considered appropriate to apply statistical comparisons to these values.

In the low-viscosity group, LCBF was increased in nearly all brain structures analyzed during increased Pa_{CO_2}. The largest increase was found mainly in cortical structures of the telencephalon, whereas the smallest increase was mainly observed in cerebellar, brainstem, and white matter structures. In contrast, no increase was found in the high-viscosity group. Thus, the values obtained for LCBF confirm what has been found for global cerebral blood flow.

Discussion

The present investigation supports the hypothesis that changes in blood viscosity do not result in changes in cerebral blood flow as long as the brain vessels can compensate for these changes by an adjustment of their flow resistance. If the brain vessels have already been dilated in response to a high blood viscosity, a hypercap-

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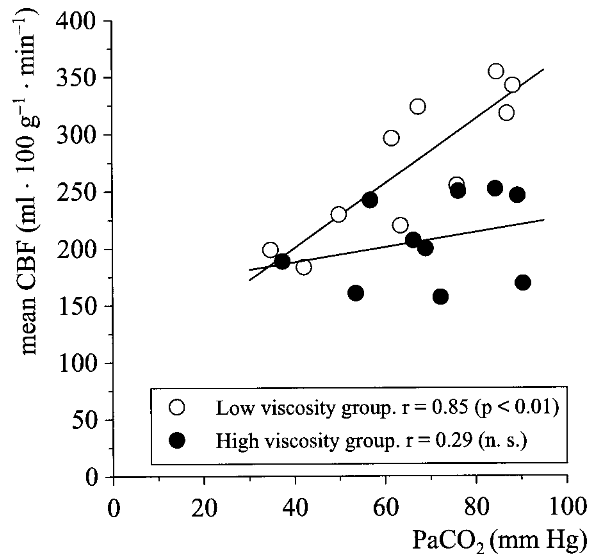

Fig. 1. Relation between mean cerebral blood flow and arterial carbon dioxide tension ($Pa{CO_2}$) after blood exchange in the low- and high-viscosity groups. Each dot represents the mean cerebral blood flow (CBF) of one rat. Whereas a normal carbon dioxide reactivity is found in the low-viscosity group, the increase in CBF is abolished in the high-viscosity group. The regression lines were calculated according to the formula $y = ax + b$, where y is mean CBF and x is $Pa{CO_2}$. Low-viscosity group (cross-linked hemoglobin [cHb]): $y = 2.8x + 92$ (slope different from zero; $P < 0.01$). High-viscosity group (cross-linked hemoglobin–polyvinylpyrrolidone [cHb-PVP]): $y = 0.7x + 162$ (slope not different from zero).

nic stimulus cannot induce a further vasodilation, resulting in a lack of a further increase in cerebral blood flow.

Vascular reactions that may be responsible for an adjustment of flow resistance after changes in blood viscosity have been measured in pial arteries and the basilar artery during hemodilution. Infusion of mannitol[18] or hemodilution[5,19] induced both a decrease of oxygen-carrying capacity and viscosity of blood because of a falling hematocrit. An increase in cerebral blood flow was verified in two of these studies by the use of a thermal diffusion probe[19] or radioactive microspheres.[5] Despite the increase in cerebral blood flow, the vessels observed were found to constrict during hemodilution. The vasoconstriction of the cerebral resistance vessels indicates a compensatory reaction which tends to reduce an increase in cerebral blood flow that would occur as a reaction to a induced decrease in blood viscosity by hemodilution. Such reactions appear to represent an adaptive mechanism of the cerebral vessels to changes in blood viscosity. However, the observations of these previous investigations were limited to easily accessible blood vessels (*e.g.,* pial vessels and basilar artery). It has been shown that it is not always possible to conclude from the behavior of these vessels to less accessible parts of the cerebral circulation.[20] The current study demonstrates that such adaptive mechanism may affect cerebral blood flow as a whole and may be exhaustible during extreme conditions.

The assumed existence of compensatory mechanisms in the cerebral circulation is further supported by three previous studies, which used radioactive microspheres to show that an increase in blood viscosity does not induce a corresponding decrease of cerebral blood flow. Chen *et al.*[21] exchanged the blood of anesthetized dogs for donor blood in which viscosity had been increased by the addition of dextran, thus maintaining a constant oxygen content of the blood. The authors reported a decrease of blood flow in the spleen and the small intestine, whereas no change of regional blood flow was observed in the heart and in the brain. Krieter *et al.*[22] infused small amounts of ultrahigh-molecular-weight dextran (4% of total blood volume) into beagle dogs. The authors obtained an increase in plasma viscosity from 1.06 to 2.99 mP · s and a reduction of the hematocrit from 36% to 20%. Despite the increase in blood viscosity,

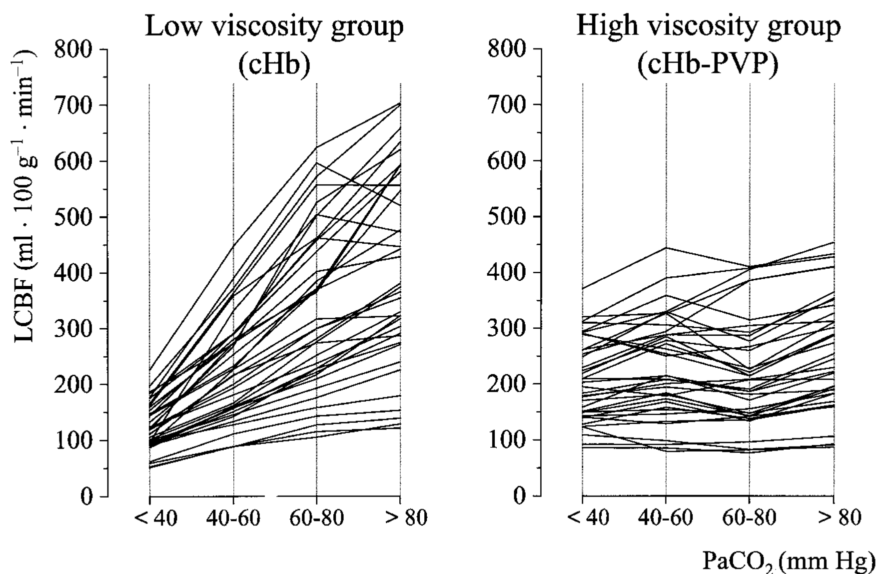

Fig. 2. Local cerebral blood flow (LCBF) in 36 individual brain structures after blood exchange during four ranges of arterial carbon dioxide tension ($Pa{CO_2}$). Each local cerebral blood flow value is the mean of all values measured in one brain structure at the corresponding range of $Pa{CO_2}$. The numbers of animals analyzed at one range of $Pa{CO_2}$ values in each of both groups were as follows: $Pa{CO_2}$ less than 40 mmHg, one rat; $Pa{CO_2}$ equal to 40–60 mmHg, two rats; $Pa{CO_2}$ equal to 60–80 mmHg, four rats; and $Pa{CO_2}$ greater than 80 mmHg, three rats. The lines joining individual data points in the graph mark LCBF values from identical brain structures in different animals during increasing stages of hypercapnia. cHb = cross-linked hemoglobin solution; PVP = polyvinylpyrrolidone.

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the researchers observed an increase in blood flow to all organs investigated, including the brain. These observations are in accordance with those of Fan *et al.,*[23] who observed that oxygen transport to brain, heart, kidney, liver, and small intestine is maintained at a constant level in anesthetized dogs when blood viscosity is either decreased by hemodilution using blood plasma or is increased by hemoconcentration using packed erythrocytes. Concerning the cerebral circulation, the findings of these three studies are confirmed by a previous investigation of our group using [^{14}C]iodoantipyrine autoradiography in rats. A near-total blood exchange by hemoglobin solutions of fourfold differing viscosities did not result in differences in cerebral blood flow at different blood viscosities.[11]

However, changes in viscosity may affect cerebral blood flow during ischemic or high-flow conditions. Cole *et al.*[24] have partly exchanged the blood of rats after a focal cerebral ischemic lesion by a diaspirin cross-linked hemoglobin solution. This treatment resulted in an increase of blood flow in the ischemic brain tissue. Because oxygen content of the blood remained unchanged after hemodilution because of the comparable oxygen-carrying capacity of the used hemoglobin solution, the authors attributed this increase of blood flow in the ischemic brain tissue to a lowering of whole-blood viscosity.[24] In analogy to the current investigation, Tomiyama *et al.*[25] recently induced a high state of brain perfusion in anesthetized rats by hypercapnia or hemodilution and increased plasma viscosity by infusion of dextrane, 9%. Using the hydrogen clearance method, these authors observed that an increased plasma viscosity induced a decrease in cerebral blood flow during extreme hypercapnia or hemodilution, whereas cerebral blood flow was only slightly decreased during normocapnia or at normal hematocrit values.[25] The findings of Tomiyama *et al.*[25] at hypercapnia and hemodilution are in accordance with the current investigation and previous observations from our group in conscious, hemodiluted rats.[26] The current study extends these findings because it shows that blood viscosity appears to influence cerebral blood flow when blood viscosity in the cerebral circulation is increased to 3.6 mP · s, which is the blood viscosity in the high-viscosity group. Conversely, at the blood viscosity in the low-viscosity group of 1.2 mP · s, compensatory mechanisms can take place (figs. 1 and 2).

It appears surprising that in both of the investigations from our group, mean blood viscosity as determined after blood exchange in the high-viscosity group is not higher, and even a bit lower, than mean viscosity of normal blood. Even at the highest shear rate the normal blood viscosity as determined in a viscosimeter is not higher in the high-viscosity group than in normal control animals. The loss of the ability to dilate to carbon dioxide in the animals of the high-viscosity group compared with normal control animals indicates that mechanisms in addition to the shear rate–dependent changes in viscosity exist in the microcirculation which keep the fluidity of normal blood high. Such mechanisms are as follows: Hematocrit, a major determinant of blood viscosity, is decreased in the microcirculation by the Fåhræus–Lindquist effect[27] to a value of approximately two-thirds of that found in the global circulation.[28–30] In addition, in cross-sections of perfused cerebral capillaries, a plasma layer of 21% of the total capillary diameter has been observed between the luminal surface of endothelial cells and each side of the erythrocyte membrane.[31] Such factors can increase the fluidity of blood in the microcirculation *in vivo*, although they cannot be detected by viscosimetric measurements *in vitro*.

Exchange transfusion using stroma-free hemoglobin solutions may be complicated by vasomotor actions of nitric oxide that is scavenged by the free hemoglobin. However, it has been shown that a possible nitric oxide scavenging effect of free hemoglobin may have only a minor impact on cerebral vasodilation. Ulatowski *et al.*[32] hemodiluted cats by either hemoglobin solution or albumin solution to a hematocrit of 16–18%. Cerebral blood flow, measured by radioactive microspheres, increased in the albumin group compared with the hemoglobin group and with a control group without hemodilution. Because nitric oxide scavenging by hemoglobin might have inhibited cerebral vasodilation, thus preventing an increase in cerebral blood flow in the hemoglobin group, the nitric oxide synthetase inhibitor *N*-ω-nitro-L-arginine methylester (L-NAME) was administered to all animals at the end of the experiment to inhibit all nitric oxide–mediated vasodilatory effects in the albumin group too. However, in the albumin group, cerebral blood flow decreased only slightly, and the difference in cerebral blood flow between the hemoglobin group and the albumin group before administration of L-NAME was only reduced by 22%. Therefore, it was concluded that the major cause of the lower blood flow observed in the hemoglobin group could not be nitric oxide scavenging, but rather, the higher blood oxygen content.[32] The minor role of nitric oxide for vasomotion during varieties in hematocrit is further supported by the observation of Todd *et al.*[33] in rabbits treated with L-NAME. These authors found that nitric oxide may be responsible for vasodilation during hypoxemia but not during hemodilution.[33] In addition, Hudetz *et al.*[34] recently used 7-nitroindazole as an inhibitor of neuronal nitric oxide synthetase during hemodilution and reported that nitric oxide may contribute to cerebral vasodilation in rat brain at intermediate levels of hemodilution, but not at those higher levels of hemodilution whose oxygen-carrying capability of blood was similar to that observed in the experimental groups of the current observation.

It has been hypothesized that nitric oxide may be a mediator for cerebrovascular dilation during hypercap-

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nia.[35,36] If the hemoglobin solution used in the current study had a nitric oxide scavenging effect and nitric oxide would significantly contribute to the hypercapnic increase in cerebral blood flow, an impairment of carbon dioxide reactivity should be observed. However, the increase of cerebral blood flow measured in the low-viscosity group of the current study during an increased Pa_{CO_2} does not show any impaired carbon dioxide reactivity of the brain vessels after blood exchange. The increase in mean cerebral blood flow of 2.8 ml · $mmHg^{-1}$ · 100 g^{-1} · min^{-1} as determined in the low-viscosity group was within the range found in previous investigations in non–blood-exchanged conscious,[37] anesthetized,[38,39] and blood-exchanged conscious rats.[40]

It cannot be excluded that some unique interaction between viscosity and hypercapnia may explain the findings of this study. However, this seems improbable because the hypothesis of this study that a hypercapnic stimulus cannot induce a further vasodilation when brain vessels have already been dilated maximally in response to a high blood viscosity is supported by recent investigations from our group in non–blood-exchanged rats.[41] In these investigations, the occlusion of both carotid arteries was used instead of hypercapnia to stimulate maximal blood vessel dilation downstream to the occlusion site. Similar to the current study, a decreased blood flow could be observed at a high blood viscosity in the affected brain regions.[41]

In the current study, the high-viscosity group showed a lower hemoglobin concentration and oxygen content than did the low-viscosity group after blood exchange. This difference may be a result of the higher oncotic pressure of the blood in the high-viscosity group. The addition of PVP (table 2) may have induced a shift of fluid from the interstitial space to the vascular space. It could be argued that this difference in circulating volume has caused the difference in mean cerebral blood flow between the two groups. However, the hemoglobin concentration and oxygen content were lower in the cHb-PVP group in which cerebral blood flow was lower than in the cHb group. If the lower hemoglobin concentration and oxygen content in the high-viscosity group had an influence on cerebral blood flow, the differences in cerebral blood flow observed between both groups during hypercapnia should have been even larger after correction for hemoglobin concentration and oxygen.

Clinical studies of the effects of blood viscosity on cerebral blood flow have been conflicting. Brown *et al.*[4] studied determinants of cerebral blood flow in patients during anemia and polycythemia. From a multiple-regression analysis of the data, it was concluded that cerebral blood flow but not blood viscosity varied inversely with arterial oxygen content. In contrast to these findings, an influence of blood viscosity on cerebral blood flow was postulated by Humphrey *et al.*[42] in paraproteinemic patients. These authors compared cerebral blood flow and blood viscosity in paraproteinemic and anemic patients in which the hematocrits were matched. In the paraproteinemic group, blood and plasma viscosity were higher and cerebral blood flow was lower than in the anemic group, suggesting that in this group of patients blood viscosity is one determinant of cerebral blood flow. However, clinical studies may be of limited value because of the heterogeneity of patient groups and the difficulties that exist when viscosimetric data obtained from peripheral blood are used.

In conclusion, the lack of increase in cerebral blood flow in the high-viscosity group during hypercapnia in the current study indicates an outstripping of the dilating mechanisms that allow maintenance of cerebral blood flow at a high blood viscosity. These data support the hypothesis that changes in blood viscosity can be compensated for by vascular adjustments in the brain during physiologic conditions, whereas such vascular adjustments may not be sufficient to maintain the oxygen supply of the brain during pathophysiologic conditions.

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