Extracellular fetal hemoglobin induces increases in glomerular permeability: inhibition with α₁-microglobulin and tempol

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Sverrisson K, Axelsson J, Rippe A, Gram M, Åkerström B, Hansson SR, Rippe B. Extracellular fetal hemoglobin induces increases in glomerular permeability: inhibition with α₁-microglobulin and tempol. Am J Physiol Renal Physiol 306:F442–F448, 2014. First published December 11, 2013; doi:10.1152/ajprenal.00502.2013.—Extracellular fetal hemoglobin (HbF) and adult hemoglobin (HbA) are proinflammatory and generate ROS. Increased plasma levels of extracellular HbF have recently been reported to occur in early pre-eclampsia. α₁-Microglobulin (A1M) is a physiological heme-binding protein and radical scavenger that has been shown to counteract vascular permeability increases induced by HbA in the perfused placenta. The present study was performed to investigate whether HbF and HbA will increase glomerular permeability in vivo and to test whether A1M and tempol, a ROS scavenger, can prevent their effects. Anesthetized Wistar rats were continuously infused intravenously with either HbA, HbF, or cyanoinactivated HbF together with FITC-Ficoll-70/400, inulin, and 51Cr-labeled EDTA for 2 h. Plasma samples and urine samples (left ureter) were taken repeatedly and analyzed by high-performance size exclusion chromatography to assess glomerular sieving coefficients for Ficoll of radius 10–80 Å. In separate experiments, A1M or tempol was given before and during Hb infusions. Extracellular HbF caused rapid, transient increases in glomerular permeability to large Ficoll molecules (50–80 Å), contrary to the effects of HbA and cyanoinactivated HbF. For HbF, glomerular sieving coefficients for Ficoll of radius 60 Å increased from 3.85 ± 0.85 × 10⁻⁵ to 2.60 ± 0.96 × 10⁻⁴ at 15 min, changes that were abrogated by tempol and reduced by A1M. In conclusion, our data demonstrate that extracellular HbF, infused systemically, can acutely increase glomerular permeability through inducing oxidative stress.

adult hemoglobin; capillary permeability; Ficoll; glomerular sieving coefficient; preeclampsia

Hemoglobin (Hb) is the major oxygen carrier of blood but has a number of toxic, potentially dangerous, side effects. Hb is a tetramer consisting of four globin subunits (α₂β₂), each carrying an iron-containing heme group in its active center (15). Most Hb is found strictly compartmentalized within erythrocytes, but during pathological hemolytic conditions, quantities of Hb leak out into the circulation. Extracellular (free) Hb and its metabolites, heme and iron, induce oxidative stress by formation of ROS (14, 18, 25), which may lead to acute renal failure and vascular dysfunction (14). In adults, the most common Hb isoform is HbA. In the fetus, the Hb molecule (fetal Hb (HbF)) is made up by two α-chains and two γ-chains (α₂γ₂). The γ-chains are gradually replaced by β-chains at birth and as the infants grow (15). HbF is a stronger binder of oxygen in vivo than HbA, because it has a lower affinity for erythrocytic 2,3-diphosphoglycerate. This facilitates oxygenation of the fetus in utero. In healthy adults, HbF is restricted to red blood cells called F-cells, which constitute a limited proportion of the total red blood cell mass (~1% of total adult Hb is HbF) (45).

Many disorders with elevated levels of HbF in adult life are known and can usually be classified as inherited or acquired. Among the inherited disorders are, for example, hereditary persistence of HbF, β-thalassemia, and sickle cell disease. Acquired conditions with elevated HbF levels include pregnancy and the pregnancy-associated disease preeclampsia (45, 51). The pathogenesis of preeclampsia is not fully understood, and the only definitive treatment is delivery of the placenta, a fact that has led to the theory of placenta-derived factors as a culprit. It is currently thought that circulating factors produced by the diseased placenta, such as antiangiogenic factors, e.g., soluble Fms-like tyrosine kinase 1 (s-Flt1) receptor (or VEGF receptor 1) (17) and soluble endoglin (32), agonistic autoantibodies to the ANG II type 1 (AT₁) receptor (48), syncytiotrophoblast membrane microparticles (19, 41), and, recently, extracellular HbF contribute (5, 16, 31, 39).

Recent studies have thus suggested that extracellular HbF is involved in the pathogenesis of preeclampsia. In 2008, Centlow et al. (16), using microarray techniques and proteomics, found an upregulation of HbF genes and accumulation of extracellular HbF in the vascular lumen in preeclamptic placentas (16). Furthermore, Olsson et al. (39) demonstrated that preeclamptic women show increased plasma levels of extracellular HbF and also of extracellular HbA (39). In preeclampsia, it thus seems that some unknown events induce local placental hypoxia, which, in turn, induces an upregulation of placental HbF genes and proteins. Extracellular HbF induces the formation of ROS, and, hence, oxidative damage and leakage of the fetomaternal barrier. This leakage results in an increased maternal plasma concentration of HbF and further induction of ROS and, as a consequence, endothelial dysfunction, hypertension, and proteinuria, which are all hallmarks of preeclampsia (39).

Olsson et al. also reported that preeclamptic women exhibit increased plasma levels of α₁-microglobulin (A1M). A1M is a 26-kDa glycoprotein mainly synthesized in the liver, secreted to the blood, and found in the interstitial fluid of all human tissues (36). A1M has been shown to have reductase activity as well as heme- and radical-binding properties (2–4, 27). The protective role of A1M in preeclampsia has been corroborated by placenta perfusion experiments (31). Furthermore, the use of A1M and extracellular HbF as early predictive and diagnostic markers for preeclampsia has turned out to be promising.
Thus, already at 10–16 wk of gestation, HbF and A1M were significantly increased in women who subsequently developed pre-eclampsia (5).

The purpose of the present study was to investigate if extracellular HbF and/or extracellular HbA can affect the permeability of the normal rat glomerular filtration barrier (GFB) in vivo, 2) to what extent the effects on GFB are ROS dependent by infusion of cyano-inactivated (CN)-HbF or the superoxide radical scavenger tempol (49), and 3) whether the adverse effects could be counteracted by A1M. For this purpose, we studied glomerular sieving coefficients (θ) of FITC-Ficoll 70/400 (molecular weights: 70,000 and 400,000, respectively) of molecular radius [Stokes-Einstein radius (aₑ)] ranging from 10 to 80 Å in rats systemically infused with HbF, HbA, or CN-HbF. Ficoll is a neutral copolymer of sucrose and epichlorohydrine, which is not (significantly) reabsorbed in the proximal tubules and therefore can serve as direct probe of glomerular permeability to macromolecules via analysis of its steady-state urine-to-plasma concentration ratio using fine-tuned high-performance size exclusion chromatography (HPSEC), which is able to detect very low θ values, five orders of magnitude lower than unity.

**MATERIALS AND METHODS**

**Animals and Surgery**

Experiments were performed in 40 male Wistar rats (Möllegard, Lille Stensved, Denmark) with an average body weight of 260.1 ± 1.2 g. Rats had free access to standard chow and water until the day of the experiment. Experiments were approved by The Animal Ethics Committee of Lund University. Rats were anesthetized using an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and anesthesia was maintained with repeated intra-arterial pentobarbital injections through the tail artery. Rats were placed on a heating pad to maintain body temperature at 37°C. The tail artery was cannulated [polyethylene (PE)-50 cannula] for monitoring of mean arterial blood pressure and heart rate on a polygraph (model 7B, Grass Instruments, Quincy, MA). A tracheotomy was performed to facilitate breathing. The left carotid artery and left and right jugular veins were cannulated (PE-50 cannulas) for blood sampling and infusions, respectively. For urine sampling, the left ureter was cannulated (PE-10 coupled to PE-50) through a small incision in the abdominal wall (6–8 mm). Furosemide (0.375 mg/kg, Furosemid, Recip) was administered via the tail artery to increase urine production and facilitate the cannulation of the ureter.

**Experimental Procedures**

**Effects of HbF, HbA, and CN-HbF.** All experiments started with an initial resting period of 20–30 min after the cannulation of the ureter, during which time a bolus and the infusion of a Ficoll-inulin-51Cr-EDTA (51Cr-EDTA) mixture (see below) was started. Immediately after the resting period, sampling of urine and plasma for glomerular Ficoll sieving measurements was performed during a 5-min control (‘baseline’) period. Subsequently, a bolus dose (61.5 μg) and a continuous infusion (3 μg/min) of either HbF (n = 8), HbA (n = 8), or CN-HbF (n = 8), respectively, was given. The Hb infusion was continued throughout the experiments. Blood and urine were sampled at 5, 15, 30, 60, and 120 min. At each sampling time, urine was collected over a 5-min period, and blood samples were collected in the middle of the urine collection period (at 2.5 min).

**Effects of A1M and tempol on HbF-induced changes in glomerular permeability.** In separate experiments, rats were pretreated with either A1M (n = 8) or the superoxide dismutase mimetic tempol (4-hydroxy-TEMPO, Sigma-Aldrich, n = 8) before being infused with HbF. The infusion of tempol (0.5 mg·kg⁻¹·min⁻¹ dissolved to 30 mg/ml) was started 5 min before the start of the HbF administration and then continued through the experiment, for a total dose of 15 mg/kg. For A1M-pretreated animals, two bolus doses of 1 mg A1M each were given 3 min apart, and an infusion of A1M (22.4 μg/min) was then started simultaneously with the HbF administration and continued throughout the experiment. The most pronounced increase in glomerular permeability after HbF occurred at 15 min (see Results), and therefore this time point was chosen to test any effects of A1M or tempol on HbF-induced increases in permeability. Blood and urine were sampled during baseline and then at 15 min after the start of the HbF administration. During each sampling period, urine was collected over a 5-min period, and blood samples were collected in the middle of the urine collection period, as described above.

**Preparation of HbF, HbA, CN-HbF, and A1M**

HbA was purified from adult whole blood, freshly drawn from healthy blood donors, whereas HbF was obtained from umbilical cord blood, as previously described (50). CN-HbF was prepared by mixing Hb (1 mM) with 10 mM KCN and then incubated for 10 min at 20°C, as previously described by Olsson et al. (38). The solution was desalted on a Sephadex G-25 column and eluted with 20 mM Tris-HCl at pH 8.0. Recombinant human A1M, containing an N-terminal His tag, was expressed in Escherichia coli and purified as previously described (26) with the addition of an ion-exchange chromatography purification step as previously described by Olsson et al. (37).

**Glomerular Permeability: Glomerular Sieving of FITC-Ficoll**

To measure glomerular permeability, the glomerular sieving of FITC-labeled Ficoll was investigated. Ficoll 70, Ficoll 400, and inulin, all labeled with FITC, were obtained from TdB Consultancy (Uppsala, Sweden). A mixture containing 960 μg FITC-Ficoll 400, 40 μg FITC-Ficoll 70, 500 μg FITC-labeled inulin, and 80 μl 51Cr-EDTA (3.7 MBq/ml) was given as a priming bolus dose followed by a constant infusion (12 ml·kg⁻¹·h⁻¹) of FITC-Ficoll 400 (0.48 mg/ml), FITC-Ficoll 70 (20 μg/ml), FITC-inulin (0.5 mg/ml), and 51Cr-EDTA (0.296 MBq/ml) for a minimum of 20 min before the start of measurements to achieve a steady-state concentration and allow animals to recover from the surgery. The infusion was then continued throughout the experiments.

Plasma and urine samples were assessed using a HPSEC (Waters, Milford, MA) with an Ultrasoundlution 500 column (Waters) and calibrated as previously described at some length (6). The mobile phase was driven by a pump (Waters 1525), and fluorescence was detected with fluorescence detector (Waters 2475) with an excitation wavelength at 492 nm and an emission wavelength at 518 nm. Samples were loaded to the system with an autosampler (Waters 717 plus), and the system was controlled by Breeze software 3.3 (Waters).

θ values were obtained by analyzing HPSEC curves from plasma and urine samples during each experiment. The θ value of Ficoll was determined as the fractional clearance according to the following formula: \(\theta = \frac{(C_{\text{FU}} \times C_{\text{IP}})}{(C_{\text{FP}} \times C_{\text{MU}})}\), where \(C_{\text{FU}}\) is the urine Ficoll concentration, \(C_{\text{IP}}\) is the inulin concentration in plasma, \(C_{\text{FP}}\) is the Ficoll concentration in plasma, and \(C_{\text{MU}}\) is the inulin concentration in urine.

**Glomerular Filtration Rate**

Glomerular filtration rate (GFR) was assessed using the steady-state clearance of 51Cr-EDTA (Amersham Biosciences, Buckinghamshire, UK) from plasma to urine. Urine was collected from the left ureter repeatedly during the experiment, and blood samples using microcapillaries (25 μl) were taken for the calculation of GFR approximately every 10–30 min. Radioactivity in blood and urine was measured using a gamma-counter (Wizard 1480, Wallac, Turku, Finland). Hematocrit was assessed throughout the experiments to...
allow the conversion of blood radioactivity into plasma radioactivity. During the FITC-Ficoll sieving periods, GFR was also assessed from the urine clearance of FITC-inulin (results not shown). GFR was calculated by dividing the urinary excretion (Ut × V_u) of 51Cr-EDTA and/or inulin per minute by the plasma tracer concentration, where U_t is the tracer concentration in urine and V_u is the flow of urine per minute.

Two-Pore Analysis

A two-pore model (30, 42) was used to analyze the θ data for Ficoll molecular radius 10–80 Å. A nonlinear least-squares regression analysis was used to obtain the best-curve fit using scaling multipliers, as previously described at some length (30). The four major parameters of the two-pore model are as follows: the small pore radius (r_s), the large pore radius (r_L), the unrestricted pore area over unit diffusion path-length (A_0/ΔX), and the fraction of the glomerular ultrafiltration coefficient accounted for by large pores (α_L). These parameters describe the membrane properties without being influenced by hemodynamic factors. r_s is determined by slope of the r_L curve (between ~50 and ~80 Å), r_L is dependent on sieving data close to the inflection point between the r_L curve and the r_s curve (between ~40 and ~46 Å). A_0/ΔX is a diffusive parameter that reflects the surface area of the small pores. α_L reflects abundance of large pores in the glomerular filter and is calculated from the fraction of GFR that is diverted through the large pores (J_GFR/vL). J_GFR/vL was obtained by extrapolating the r_L curve back to the ordinate (i.e., to 0 Å).

Statistics

Values are presented as means ± SE. Differences among groups were tested using nonparametric ANOVA with the Kruskal-Wallis test and post hoc testing used the Mann-Whitney U-test. Bonferroni corrections for multiple comparisons were made when applicable. Significant levels were set at P < 0.05. All statistical calculations were made using IBM SPSS statistics (version 20.0.0) for Windows (SPSS, Chicago, IL).

RESULTS

θ Values During Systemic Infusion of HbF, HbA, or CN-HbF

Figure 1 shows θ values plotted versus α_L for Ficoll of radius 10–80 Å during baseline and after 15 min of systemic infusion of HbF, HbA, or CN-HbF, respectively. In the α_L range of 60–80 Å, there was a marked increase in θ values for animals infused with HbF but not for animals infused with HbA or CN-HbF. θ values for Ficoll of radius 60 Å thus increased from 3.85 ± 0.85 × 10^−5 to 2.60 ± 0.96 × 10^−4 (P < 0.05) and that for Ficoll of radius 80 Å from 3.16 ± 0.78 × 10^−5 to 2.39 ± 0.92 × 10^−4 in HbF-infused animals (P < 0.05).

In Fig. 2, θ value changes for Ficoll of radius 60 Å are shown as a function of time. Already at 5 min, there was a marked increase in glomerular permeability for animals infused with HbF, which peaked at 15 min but reversed within 120 min. In contrast, θ values for HbA and CN-HbF did not change significantly over time.

Pretreatment With A1M and Tempol

In animals pretreated with tempol or A1M, there were no significant increases in glomerular permeability after HbF infusion compared with baseline, as shown in Fig. 3. Thus, tempol more or less totally abrogated and A1M markedly reduced HbF-induced changes in glomerular permeability.

Two-Pore Modeling

The best-curve fits of θ values versus α_L for Ficoll according to the two-pore model were obtained using the parameters...
shown in Table 1 for animals treated with HbF and HbA after 15, 30, and 120 min. αL increased significantly (4-fold) at 5 min (P < 0.05), 15 min (P < 0.05), and 30 min (P < 0.01) compared with baseline. Similarly, JvL/GFR increased at 5, 15, and 30 min. Furthermore, there was an increase in rL over time (P < 0.05), which, however, only reached significance at 30 min. A0/ΔX and rS did not change in any of the groups. For HbA and CN-HbF, there were no statistically significant changes in pore parameters over time for either animals treated with A1M or tempol (data not shown).

Hemodynamic Parameters

None of the groups had any significant changes in heart rate (not shown) or mean arterial pressure (Fig. 4) over time.

GFR

GFR (Fig. 5, A and B) remained more or less stable in all groups except for HbF-infused animals treated with A1M. In this group, there was a slight but significant (P = 0.036), decrease in GFR, from 0.82 ± 0.06 ml-min⁻¹-g⁻¹ at baseline to 0.63 ± 0.06 ml-min⁻¹-g⁻¹ at 15 min.

DISCUSSION

The essential result of this study is that systemic infusions of relatively low concentrations of extracellular HbF into healthy anaesthetized rats were able to markedly and transiently increase glomerular permeability to macromolecules. This effect could be inhibited by A1M, a physiological heme-binding protein and radical scavenger, and by the ROS scavenger tempol.

Table 1. Two-pore parameters for HbF and HbA at baseline and at 15, 30, and 120 min of Hb infusion

<table>
<thead>
<tr>
<th>Two-pore Parameter</th>
<th>Baseline</th>
<th>15 min</th>
<th>30 min</th>
<th>120 min</th>
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<tbody>
<tr>
<td></td>
<td>HbF</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>rS, Å</td>
<td>44.58 ± 0.51</td>
<td>44.74 ± 0.49</td>
<td>44.89 ± 0.46</td>
<td>44.53 ± 0.31</td>
</tr>
<tr>
<td>rL, Å</td>
<td>131.64 ± 8.45</td>
<td>196.00 ± 24.75</td>
<td>186.97 ± 11.29*</td>
<td>126.07 ± 9.29</td>
</tr>
<tr>
<td>A0/ΔX, cm × 10⁻⁵</td>
<td>7.24 ± 1.01</td>
<td>6.09 ± 0.76</td>
<td>6.41 ± 0.81</td>
<td>7.44 ± 0.95</td>
</tr>
<tr>
<td>JvL/GFR, ×10⁵</td>
<td>8.09 ± 1.26</td>
<td>35.11 ± 10.65*</td>
<td>26.81 ± 4.43†</td>
<td>9.23 ± 1.93</td>
</tr>
<tr>
<td>αL, ×10⁵</td>
<td>2.51 ± 0.38</td>
<td>9.34 ± 2.66*</td>
<td>7.29 ± 1.14†</td>
<td>2.82 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>HbA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rS, Å</td>
<td>44.91 ± 0.30</td>
<td>45.80 ± 0.28</td>
<td>45.74 ± 0.27</td>
<td>45.05 ± 0.33</td>
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<tr>
<td>rL, Å</td>
<td>133.53 ± 9.71</td>
<td>130.63 ± 10.56</td>
<td>152.49 ± 2.44</td>
<td>126.86 ± 4.46</td>
</tr>
<tr>
<td>A0/ΔX, cm × 10⁻⁵</td>
<td>7.39 ± 0.59</td>
<td>6.10 ± 1.28</td>
<td>5.34 ± 0.55</td>
<td>7.48 ± 1.09</td>
</tr>
<tr>
<td>JvL/GFR, ×10⁵</td>
<td>8.10 ± 0.86</td>
<td>12.29 ± 2.34</td>
<td>15.14 ± 4.15</td>
<td>9.83 ± 2.25</td>
</tr>
<tr>
<td>αL, ×10⁵</td>
<td>2.53 ± 0.28</td>
<td>3.79 ± 0.86</td>
<td>4.33 ± 1.16</td>
<td>3.04 ± 0.69</td>
</tr>
</tbody>
</table>

Values are means ± SE. HbF, fetal hemoglobin (Hb); HbA, adult Hb; rS, small-pore radius; rL, large-pore radius; A0/ΔX, effective pore area over unit diffusion path-length; JvL/GFR, fractional fluid flow through large pores (where GFR is the glomerular filtration rate); αL, fractional ultrafiltration coefficient accounted by large pores. Statistical differences between test situations and baseline: *P < 0.05 and †P < 0.01.

Fig. 4. Mean arterial pressure (MAP) as function of time for animals infused with HbF, HbA, and CN-HbF, respectively. MAP values for A1M- and tempol-treated animals are not shown. There were no significant changes in MAP over time.

Fig. 5. A: glomerular filtration rate (GFR) as a function of time for animals infused with HbA, HbF, and CN-HbF, respectively. There were no significant changes in GFR over time in these experiments. B: GFR as a function of time for HbF-infused animals treated with A1M or tempol compared with HbF-infused animals. The slight decrease in the group treated with A1M after 15 min was statistically significant compared with baseline (*P < 0.05).
reduced to superoxide. In the next step, ferryl (Fe4OxyHb, i.e., ferrous Hb (Fe2+ to be toxic because of their oxidative properties (14, 18). Extracellular Hb and its metabolites, heme and iron, are known to be toxic because of their oxidative properties (14, 18). OxyHb, i.e., ferrous Hb (Fe2+) and oxygen, can undergo spontaneous intramolecular oxidation-reduction reactions, in which iron is oxidized to its ferric (Fe3+) form and oxygen is reduced to superoxide. In the next step, ferryl (Fe4+) Hb, free heme, and various ROS, including hydroxyl radicals, superoxide, and hydrogen peroxide, can be formed. All these compounds may cause oxidative damage to cell membranes, DNA, and other tissue components, scavenge nitric oxide (NO), and uncouple endothelial NO synthase to induce further ROS production and reduce the bioavailability of NO (18). In turn, scavenging of NO may lead to elevations in blood pressure and increases in vascular permeability, signs of endothelial dysfunction (24). The dose of HbF given was not large enough to reveal any substantial systemic scavenger effects on NO, because it caused no significant changes in mean arterial pressure or GFR.

In plasma, free Hb and heme are detoxified by binding to haptoglobin (Hp), albumin, and hemopexin (25). The CD163 receptor on macrophages scavenges the Hp-Hb complex and CD91 receptor on several cell types and can scavenge the Hp-heme complex (23). Furthermore, heme oxygenase is an important intracellular heme degradation enzyme (25). The endogenous protein A1M is a plasma and tissue protein that has been shown to be involved in the defense against cell-free Hb and heme and is found in the extravascular space either as a free molecule or as a high-molecular-weight complex bound to IgA, albumin, or prothrombin (13). The physiological function of A1M is probably to protect cells and tissues against oxidative stress induced by extracellular Hb and other sources of free radicals and oxidants (36). Recent studies (37, 40) have demonstrated that A1M protects cell cultures and skin explants against oxidative damage. Indeed, in the present study, A1M showed an antioxidant capacity mimicking that of the superoxide dismutase mimetic tempol.

In a previous ex vivo dual-perfusion study (31) of the placenta obtained directly after delivery, extracellular Hb (HbA) was added to the fetal part of the circulation in high (mg/ml) doses, which caused an increased perfusion pressure, fetomaternal Hb leakage, and ultrastructural changes of cells and the extracellular matrix mimicking those found in preeclampsia. Furthermore, as in preeclamptic placentas, gene array analysis showed an upregulation of genes related to apoptosis and oxidative stress. In the present experiments, much lower (μg/ml) doses of systemic free Hb were tested in intact rats in vivo. Despite the low doses, HbF was still able to produce very marked increases in glomerular permeability, whereas similar low doses of HbA failed to do so. It should, in this context, be pointed out that our model for assessing glomerular permeability has proven to be highly sensitive to a number of different systemic challenges, such as moderate trauma (7), oxidative stress (43), hyperglycemia (9), or systemic infusions of ANG II (8) and atrial natriuretic peptide (10). These permeability changes, reflected by size-selective changes in the GFB, with increases in permeability for high-molecular-weight Ficoll and albumin, have turned out to be rapid, dynamic, and usually transient in nature.

The transient effects of HbF on glomerular permeability, although HbF was continuously infused, are consistent with previous experiments on peripheral endothelial and glomerular barrier permeability in response to various permeability agonists, such as histamine, bradykinin, and thrombin. The time cycles of these permeability shifts have been found to vary from 10–20 min (histamine and bradykinin) to up to 1–2 h (thrombin) (1, 8–10, 21, 33, 52). These differences in permeability cycle times are conceivably due to differences in adaptive responses. A number of processes responsible for the restoration of barrier integrity during ongoing stimulation have been described, such as receptor desensitization, activation of signaling pathways opposing endothelial retraction, activation of small GTPases (for reassembly of adherens junctions), release of endothelial barrier-stabilizing mediators, etc. (29, 33). Increases in endogenous scavengers and detoxifying systems could also be involved, such as upregulation of heme oxygenase and ferritin, although previous studies (12, 34) have indicated that it usually takes over 1–2 h after permeability induction for heme oxygenase and ferritin activity to increase. It is speculated, though, that bouts of permeability activation, in the present case the oxidative action of HbF, would also increase glomerular permeability in the long term. Preliminary results from ongoing studies (unpublished observations) point in this direction, but this will be dealt with in a forthcoming publication.

The potent, but transient, effect of HbF, but not of HbA or CN-HbF, on glomerular permeability, fully reversible by ROS scavenging, thus indicates that HbF is a potent inducer of ROS in vivo, more potent than HbA tested earlier. Under normal physiological conditions, there is always some minor physiological hemolysis, resulting in extravascular HbA (46). It can thus be speculated that a greater number of endogenous scavengers and detoxifying systems have evolved for extracellular HbA than for HbF, preventing their deleterious effects. Conceivably, an increased presence of endogenous scavengers for HbA, but less so for HbF, in our in vivo study and, as mentioned above, a lower HbA dose than tested earlier, may be responsible for the relative lack of toxicity of HbA compared with that found in the ex vivo perfusion study (31). In the latter study, HbA caused permanent tissue damage. In the present study, in contrast, the permeability actions of HbF were completely reversible. This seems to contradict the notion of an irreversible action of HbF on glomerular structure, at least in the acute setting. Classic morphological preeclamptic alterations of the GFB, so-called endotheliosis, include endothelial cell swelling and loss of endothelial fenestration and, in some instances, occlusion of capillary lumens (47). These changes have been assigned to circulating anti-angiogenic factors, most notably the s-Fli1 receptor (soluble VEGF receptor 1) and soluble en-
doglin. It is usually put forward as an example of GFB alterations induced at the endothelial side of the glomerular filter (20). In the present experiments, we observed dynamic, transient permeability changes that can be functionally ascribed to an increased number of unselective large pores in the GFB without any significant changes in the small pore pathway. It thus seems that HbF in the acute setting, conceivably by directly interacting with the endothelium via ROS and/or the NO system, caused a direct effect on the endothelial contractile cytoskeleton. In addition, preeclampsia has also been implicated to cause reorganization of podocytes (22, 28). However, we (30) have previously shown that the critical sieving barrier to proteins in the GFB cannot be located at the podocyte level. Rather, it is likely that podocytes, via its cytoskeletal actin dynamics, affect uphill components of the GFB to produce changes in the permeability of the entire GFB.

In this study, we measured θ values for high-molecular-weight FITC-Ficoll to assess the permeability of the rat GFB. We have previously measured θ values for 125I-labeled albumin using a tissue uptake technique in parallel with these determinations. Unfortunately, however, due to high levels of denatured protein and free iodine (125I) in the current albumin preparations, we were not able to use this technique in the present study. However, in a number of previous experiments in which increased glomerular permeability has been assessed, there has been a near-complete coupling between alterations in θ values for albumin and those for high-molecular-weight Ficoll (Ficoll with radius of 50–80 Å) (7, 11, 43, 44). It is also well documented from other investigators that the θ value for albumin is well reflected by the value for Ficoll of radius 55 Å (35). Thus, we consider it safe to rely upon θ values for high-molecular-weight Ficoll as indicators of glomerular permeability and albuminuria.

A number of previous studies have focused on placental circulating antiangiogenic factors, most notably the s-Fit1 receptor, as tentative agents producing hypertension and increased microvascular permeability and proteinuria in preeclampsia. In the present study, we demonstrate that oxygen-carrying HbF, but not CN-HbF, can promptly and transiently increase glomerular permeability to macromolecules, when given systemically, and that the endogenous antioxidant A1M markedly reduced this effect. Our data thus support the theory that extracellular HbF may contribute to the proteinuria occurring in preeclampsia and that it exerts this effect by inducing oxidative stress.

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DISCLOSURES

S. R. Hansson, M. G. Olsson, and B. Åkerström hold patents related to the prediction, diagnosis, and treatment of preeclampsia.

AUTHOR CONTRIBUTIONS


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