Role of heme oxygenase-2 in pial arteriolar response to acetylcholine in mice with and without transfusion of cell-free hemoglobin polymers

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Qin X, Kwansa H, Bucci E, Doré S, Boehning D, Shugar D, Koehler RC. Role of heme oxygenase-2 in pial arteriolar response to acetylcholine in mice with and without transfusion of cell-free hemoglobin polymers. Am J Physiol Regul Integr Comp Physiol 295: R498–R504, 2008. First published May 21, 2008; doi:10.1152/ajpregu.00188.2008.—Carbon monoxide derived from heme oxygenase (HO) may participate in cerebrovascular regulation under specific circumstances. Previous work has shown that HO contributes to feline pial arteriolar dilation to acetylcholine after transfusion of a cell-free polymeric hemoglobin oxygen carrier. The role of constitutive HO2 in the pial arteriolar dilatory response to acetylcholine was determined by using 1) HO2-null mice (HO2−/−), 2) the HO inhibitor tin protoporphyrin IX (SnPPIX), and 3) 4,5,6,7-tetramethoxybenzotriazole (TBB), an inhibitor of casein kinase-2 (CK2)-dependent phosphorylation of HO2. In anesthetized mice, superfusion of a cranial window with SnPPIX decreased arteriolar dilation produced by 10 μM acetylcholine by 51%. After partial polymeric hemoglobin exchange transfusion, the acetylcholine response was normal but was reduced 72% by SnPPIX and 95% by TBB. In HO2−/− mice, the acetylcholine response was modestly reduced by 14% compared with control mice and was unaffected by SnPPIX. After hemoglobin transfusion in HO2−/− mice, acetylcholine responses were also unaffected by SnPPIX and TBB. In contrast, nitric oxide synthase inhibition completely blocked the acetylcholine response in HO2−/− mice. We conclude 1) that HO2 activity partially contributes to acetylcholine-induced pial arteriolar dilation in mice, 2) that this contribution is augmented in the presence of a plasma-based hemoglobin polymer and appears to depend on a CK2 kinase mechanism, 3) that nitric oxide synthase activity rather than HO1 activity contributes to the acetylcholine reactivity in HO2−/− mice, and 4) that plasma-based polymeric hemoglobin does not scavenge all of the nitric oxide generated by cerebrovascular acetylcholine stimulation.

blood substitutes; cerebral artery; cerebral circulation; carbon monoxide; endothelium-dependent dilation; nitric oxide

Metabolism of heme by heme oxygenase (HO) generates biliverdin and carbon monoxide (CO), which can produce vasodilation by increasing cGMP and by acting directly on calcium-sensitive potassium (KCa) channels (7, 14). A physiological role of CO generated from HO2, the constitutive isoform of HO, has been demonstrated in the central and peripheral nervous system (1, 4). However, a role for HO2 in cerebrovascular regulation has been largely limited to studies in newborn pigs. In piglets, CO has been shown to contribute to pial arteriolar dilation in response to glutamate, NMDA receptor activation, seizures, hypoxia, and arterial hypotension (6, 15, 16, 30). These effects of CO have been attributed to HO2 primarily located in cerebral endothelium and astrocytes (9, 19, 20, 25). In adult rats, cerebral vasodilation in response to severe seizures also appears to depend on HO activity (24). Although CO can dilate pial arterioles in adult rats (12), less is known about the role of HO2 in endothelium-dependent dilation of cerebral arterioles in adult animals.

Ordinarily, endothelium-dependent dilation to acetylcholine (ACH) is mediated by nitric oxide (NO) in adults. However, an NO-independent component of ACH-induced dilation requiring HO activity has been described in porcine pulmonary arteries (34). Moreover, our previous work (28) demonstrated that ACH dilation of pial arterioles in cats was blocked not only by the nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine (L-NNA), but also by the HO inhibitor tin protoporphyrin IX (SnPPIX) after an exchange transfusion of cell-free polymeric hemoglobin (Hb). We also found similar results in rats with ADP-evoked dilation (28). Thus vasodilator agonists that normally require NOS activity now require both NOS and HO activity in the presence of this plasma-based O2 carrier. This Hb polymer, with an average molecular mass of 20 MDa and with small polymers removed by 300-kDa filtration, does not readily extravasate (21) and is unlikely to scavenge significant amounts of NO in the brain interstitial space. Although the Hb polymer is stable, it is possible that a small amount of the infused protein could be taken up into the endothelium. Dependence of the dilator response on HO activity may be the result of dissociation of heme from the Hb polymer, which could provide increased substrate for the constitutive HO2 and/or cause induction of HO1. An alternative explanation is that posttranslation modification of HO2 leads to its increased activity. In contrast to HO1, HO2 is phosphorylated by a casein kinase-2 (CK2) kinase-dependent mechanism, and the CK2 inhibitor 4,5,6,7-tetramethoxybenzotriazole (TBB) blocks HO2 phosphorylation in hippocampal and olfactory neurons and inhibits HO2-dependent relaxation of internal anal sphincter muscle (4). Thus HO2 activity can be dynamically regulated and is not dependent merely on its basal constitutive activity (5).

The purpose of the present study was to test the role of HO2 in the pial arteriolar dilation response to ACh after Hb transfusion by comparing the effect of SnPPIX, a non-isozyme-specific HO inhibitor, in wild-type (HO2+/+) mice expressing...
HO2 and in HO2-null (HO2−/−) mice. Residual effects of SnPPIX in HO2−/− mice could implicate a role for HO1. We tested the hypothesis that SnPPIX decreases ACh-evoked vasodilation after exchange transfusion of the nonextravasating Hb polymer in HO2+/− mice but not in HO2−/− mice. We further tested the hypothesis that the CK2 kinase inhibitor TBB also decreases the ACh response in HO2+/− mice but not in HO2−/− mice after Hb transfusion. Finally, we tested whether the residual response in HO2−/− mice remains dependent on NOS activity.

METHODS

All procedures on mice were approved by the Johns Hopkins University Animal Care and Use Committee. HO2−/− mice were originally made by S. Tonegawa (26) and were backcrossed with C57BL/6 mice for >10 generations. Mice were anesthetized with halothane and mechanically ventilated through a tracheostomy. A catheter was inserted in a femoral artery for measuring arterial blood pressure, arterial blood gases, Hb concentration, and hematocrit. Rectal temperature was maintained at ~37°C with a heating blanket and a lamp. A closed cranial window was constructed over the parietal cortex for measuring pial arteriolar diameter by intravital microscopy (11, 27). A plastic ring was cemented to the skull, and a 3-mm craniotomy was performed within the ring. The dura was gently retracted, the ring was filled with artificial cerebrospinal fluid (CSF), and the window was sealed with a glass coverslip that was glued to the ring. The ring was equipped with a thermistor for monitoring fluid temperature, a side port for measuring fluid pressure, and inflow and outflow ports. After the surgery was completed, halothane anesthesia was discontinued and chloralose was infused through an intraperitoneal catheter with a loading dose of 30 mg/kg and a maintenance dose of 25 mg·kg−1·h−1 for the remainder of the experiment.

Bovine Hb was chemically modified to stabilize the tetramers and inhibit NOS activity (28). Because SnPPIX is photosensitive (33), care was taken to protect the freshly prepared solution from light by wrapping the infusion syringe and catheter in opaque material, by adding carbon black to the acrylic cement surrounding the cranial window, and by keeping the window covered during SnPPIX superfusion when not measuring pial arteriolar diameter. TBB was superfused at a concentration of 10 μM. Among 33 kinases that were screened, this concentration of TBB was found to be highly selective for inhibiting CK2 (32). This concentration was also found to inhibit HO2 phosphorylation and subsequent activity (4).

Eight groups of mice were studied: 1) HO2+/+ mice (n = 6) in which an ACh dose response was obtained twice to serve as a time control for repeated ACh exposure, 2) HO2+/− mice (n = 6) in which an ACh dose response was obtained before and after superfusion of the window with SnPPIX, 3) HO2+/+ mice (n = 6) that were first exchange-transfused with polymeric Hb and in which an ACh dose response was obtained before and after superfusion of the window with SnPPIX, 4) HO2−/− mice (n = 5) that were first exchange-transfused with polymeric Hb and in which an ACh dose response was obtained before and after superfusion of the window with TBB, 5) HO2−/− mice (n = 6) in which an ACh dose response was obtained before and after superfusion of the window with SnPPIX, 6) HO2−/− mice (n = 7) that were first exchange-transfused with polymeric Hb and in which an ACh dose response was obtained before and after superfusion of the window with SnPPIX, 7) HO2−/− mice (n = 5) that were first exchange-transfused with polymeric Hb and in which an ACh dose response was obtained before and after superfusion of the window with l-NNA. Comparisons of the percent dilatation at each dose of ACh in the presence of CSF or inhibitor were made among the eight groups of mice by analysis of variance (ANOVA) and the Newman-Keuls multiple range test at the 0.05 level of significance. Comparisons of the response at a particular ACh dose before and after superfusion of an inhibitor were made by paired t-test. Values are presented as means ± SD.

RESULTS

Mice that underwent an exchange transfusion with the cell-free polymeric Hb had ~30% decrease in hematocrit but only ~15% decrease in arterial blood Hb concentration (Table 1). Mean arterial blood pressure was unchanged after the transfusion in HO2+/+ and HO2−/− mice. Mean arterial blood pressure and arterial PCO2 were in the physiological range and remained stable from before the first ACh dose response until after the second ACh dose response in all transfused and nontransfused groups (Table 2). Baseline diameter of pial arterioles was decreased 60 min after Hb exchange transfusion (before exposure to ACh). The diameter change in HO2−/− mice (~4.9 ± 3.9%) was similar to that in HO2+/+ mice (~5.9 ± 3.4%). The decrease in diameter has been shown to offset the decrease in blood viscosity after Hb exchange transfusion and to maintain cerebrovascular resistance (29).

SnPPIX has good selectivity for inhibiting HO over NOS and soluble guanylyl cyclase (34), and several studies have confirmed its selectivity in HO2−/− mice (4, 8, 10). SnPPIX was superfused through the cranial window at a concentration of 10 μM, which was previously found to inhibit ACh dilatation selectively in Hb-transfused cats but not to inhibit NOS activity (28). Because SnPPIX is photosensitive (33), care was taken to protect the freshly prepared solution from light by wrapping the infusion syringe and catheter in opaque material, by adding carbon black to the acrylic cement surrounding the cranial window, and by keeping the window covered during SnPPIX superfusion when not measuring pial arteriolar diameter. TBB was superfused at a concentration of 10 μM. Among 33 kinases that were screened, this concentration of TBB was found to be highly selective for inhibiting CK2 (32). This concentration was also found to inhibit HO2 phosphorylation and subsequent activity (4).
a response that would be too small to compare statistically among groups. The order of exposure to the 3 or 10 μM concentration did not significantly affect the magnitude of the response to each dose. In the first HO2+/+ group of mice, the cortical surface was exposed to 3 and 10 μM of ACh a second time after an intervening period of 30 min following the washout from the previous ACh exposure. This group acted as a time-control group. The dilation to 3 and 10 μM of ACh during the second exposure was attenuated compared with the corresponding dilation during the first exposure (Fig. 1). Therefore, comparisons among groups required separate statistical analysis of the first and second ACh dose responses.

To determine the effect of Hb transfusion and the effect of HO2 gene deletion on the ACh response before exposure to inhibitors, groups with common treatments during the first ACh dose response were pooled (Fig. 2). With data from both ACh doses, multivariate two-way ANOVA was performed with mouse genotype and Hb transfusion as fixed effects. There was no overall effect of Hb transfusion or interaction of Hb transfusion with mouse genotype. Thus, in agreement with data in cats (28), transfusion of Hb in mice did not appreciably affect the dilator response to ACh. However, there was an overall effect of mouse genotype (P < 0.025). Univariate two-way ANOVA at each individual dose indicated a marginal effect of genotype at 3 μM (P < 0.07) and a highly significant effect of genotype at 10 μM (P < 0.01). After the nontransfused and Hb-transfused mice for each genotype were combined, the ACh dilator response at 10 μM was found to be significantly decreased by 14% from 18.6 ± 3.9% in 23 HO2+/+ mice to 16.0 ± 2.7% in 23 HO2−/− mice.

Dilator responses to ACh obtained after superfusion of SnPPIX are shown in Fig. 3. In HO2+/+ mice, SnPPIX moderately decreased the dilator response by 52% at 3 μM and by 51% at 10 μM, compared with the time-control group superfused with CSF before the second ACh exposure (Fig. 3A). In Hb-transfused HO2+/+ mice, SnPPIX markedly decreased the dilator response by 82% at 3 μM and by 72% at 10 μM, compared with the time-control group superfused with CSF before the ACh exposure. The dilator response in the Hb-transfused HO2+/+ group treated with SnPPIX was significantly less than that in the nontransfused HO2+/+ group treated with SnPPIX. Thus the inhibitory effect of SnPPIX was enhanced in the group transfused with Hb.

In HO2−/− mice treated with SnPPIX (Fig. 3B), the response to ACh was greater than in HO2+/+ mice treated with SnPPIX (Fig. 3A), thereby implying that the inhibitory effect of SnPPIX in HO2+/+ mice was mediated by HO2 activity rather than by HO1 activity. Moreover, SnPPIX failed to inhibit the responses in Hb-transfused HO2−/− mice, which also implies that the augmented inhibitory effect of SnPPIX seen in Hb-transfused HO2+/+ mice was mediated by HO2 activity. Furthermore, the intact responses of both HO2−/− groups after SnPPIX treatment indicate that upregulation of HO1 activity is not responsible for the residual vasodilator responses in the HO2−/− groups.

To determine whether CK2-mediated phosphorylation is responsible for the HO2 dependence of the ACh response after Hb transfusion, TBB was superfused in HO2+/+ and HO2−/− mice transfused with Hb. These groups had intact vasodilator responses during the initial dose response to ACh (Fig. 4). After superfusion of TBB, the responses to both doses of ACh were blocked in the HO2+/+ group transfused with Hb. However, the responses to ACh were not impaired by TBB in HO2−/− mice transfused with Hb.

In addition to the HO dependence of the ACh dilator response, the response is largely dependent on NO (23, 31) and remains dependent on NO after Hb transfusion (2, 28). To determine whether the response remains dependent on NO in HO2−/− mice after Hb transfusion, L-NNA was superfused after the initial ACh response was tested. The initial ACh dose

### Table 1. Effect of exchange transfusion on arterial hematocrit and hemoglobin concentration

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Hematocrit, %</th>
<th>Hemoglobin, g/dl</th>
<th>MABP, mmHg</th>
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<td></td>
<td></td>
<td>Pretransfusion</td>
<td>Posttransfusion</td>
<td>Pretransfusion</td>
</tr>
<tr>
<td>HO2+/+</td>
<td>CSF/SnPPIX</td>
<td>42±2</td>
<td>27±2</td>
<td>12.4±0.8</td>
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<td>HO2+/+</td>
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<td>29±2</td>
<td>11.9±1.3</td>
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<tr>
<td>HO2−/−</td>
<td>CSF/SnPPIX</td>
<td>40±4</td>
<td>28±2</td>
<td>11.5±0.9</td>
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<tr>
<td>HO2−/−</td>
<td>CSF/TBB</td>
<td>42±3</td>
<td>30±2</td>
<td>12.2±1.2</td>
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<tr>
<td>HO2−/−</td>
<td>CSF/L-NNA</td>
<td>41±5</td>
<td>29±6</td>
<td>11.6±2.3</td>
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</tbody>
</table>

Values are means ± SD. Treatments indicate artificial cerebrospinal fluid (CSF) or drug superfusion during 1st and 2nd acetylcholine dose exposures. MABP, mean arterial blood pressure; HO2, heme oxygenase-2; SnPPIX, tin protoporphyrin IX; TBB, 4,5,6,7-tetrabromobenzotriazole; L-NNA, Nω-nitro-L-arginine.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>MABP, mmHg</th>
<th>Arterial Pco2, mmHg</th>
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<td></td>
<td></td>
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<td>End</td>
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<tr>
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<td>Hb-CSF/TBB</td>
<td>96±11</td>
<td>99±17</td>
</tr>
<tr>
<td>HO2−/−</td>
<td>Hb-CSF/L-NNA</td>
<td>95±7</td>
<td>95±9</td>
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</tbody>
</table>

Values are means ± SD. Treatments indicate CSF or drug superfusion during 1st and 2nd acetylcholine dose exposures. Hb, hemoglobin.

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response in this group (Fig. 5) was similar to that in the other
HO2−/− groups. After superfusion of L-NNA, the dilator re-
sponse to ACh was abolished in HO2−/− mice transfused with
Hb (Fig. 5).

DISCUSSION

The major findings of this study are 1) that ACh-evoked
dilation of mouse pial arterioles is partially dependent on HO
activity, as evident from the 51% inhibition by SnPPIX in
HO2−/− mice but not in HO2−/− mice, 2) that this dependence
is augmented in the presence of a cell-free Hb O2 carrier and
appears to require CK2 kinase activity, and 3) that with
lifelong absence of HO2, NOS activity rather than HO1 activ-
ity sustains ACh vasoreactivity at a level slightly less than that
seen with normal HO2 expression. Together, these results
imply that HO2 is the primary isoform contributing to ACh-
evoked cerebrovascular dilation after Hb transfusion and that
plasma-based polymeric Hb does not scavenge all of the NO
generated by endothelial muscarinic stimulation in the cerebral
circulation.

Role of HO2 without Hb transfusion. The dilator response to
ACh in mouse pial arterioles normally depends on NOS
activity, as evident from the 51% inhibition by SnPPIX in
HO2−/− mice but not in HO2−/− mice, 2) that this dependence
is augmented in the presence of a cell-free Hb O2 carrier and
appears to require CK2 kinase activity, and 3) that with
lifelong absence of HO2, NOS activity rather than HO1 activ-
ity sustains ACh vasoreactivity at a level slightly less than that
seen with normal HO2 expression. Together, these results
imply that HO2 is the primary isoform contributing to ACh-
evoked cerebrovascular dilation after Hb transfusion and that

Fig. 1. Percent change in pial arteriolar diameter (±SD) during the 1st and 2nd
exposures to 3 and 10 μM acetylcholine (ACh) in a nontransfused control
group of heme oxygenase-2-expressing (HO2+/+) mice (n = 6). *P < 0.05
from 1st ACh trial at the corresponding dose by paired t-test.

Fig. 2. Percent change in pial arteriolar diameter (±SD) during the 1st
exposure to 3 and 10 μM ACh. Mice from different groups with common
treatments and no drug inhibitors during the 1st ACh exposure were pooled
into HO2+/+ cohorts without hemoglobin (Hb) transfusion (n = 12) and into HO2-null (HO2−/−) cohorts without Hb
transfusion (n = 6) and with Hb transfusion (n = 17). *Overall effect of mouse
genotype (P < 0.01) by 2-way ANOVA with genotype as 1 factor and Hb
transfusion as a second factor.

Fig. 3. Percent change in pial arteriolar diameter (±SD) to 3 and 10 μM ACh
with or without 10 μM tin protoporphyrin IX (SnPPIX) superfusion and with
or without Hb transfusion (n = 6/group). A: responses in HO2+/+ mice. B: responses in HO2−/− mice. *P < 0.05 from the nontransfused group that
was superfused with artificial cerebrospinal fluid (CSF). †P < 0.05 between
the nontransfused and Hb-transfused groups superfused with SnPPIX.
HO2 or induce HO1. However, induction of HO1 is not a likely oxygenation that leads to a shift in signaling pathways. The release of heme could provide increased substrate for HO1 expression in cats 48 h after Hb transfusion when SnPPIX remained effective in inhibiting ACh-evoked dilation (28). The lack of effect of SnPPIX on the ACh response in HO2−/− mice after Hb transfusion indicates that HO1 activity was not being rapidly upregulated and contributing to the ACh response.

An increase in heme availability is not the sole mechanism for an increased HO contribution to the ACh vascular response. The pial arteriolar dilation to ACh after Hb transfusion was completely blocked by TBB, which is a selective inhibitor of CK2 (32). Although CK2 has many target proteins (22), the lack of effect of TBB on the ACh response in HO2−/− mice implies that its actions are specific for inhibition of CK2’s effect on HO2. Because TBB blocks phosphorylation of HO2 (4) and because TBB had no effect on the ACh response in HO2−/− mice, an increase in HO2 phosphorylation appears to be necessary for the ACh response after Hb transfusion in HO2−/− mice. Therefore, the increased contribution of HO2 activity to the ACh vascular response is not merely secondary to a passive increase in heme metabolism, but represents an actively regulated response.

The mechanism for triggering CK2-dependent phosphorylation of HO2 remains to be elucidated. In neurons, PKC can phosphorylate CK2 and lead to an increase in kinase activity of CK2 (4). Whether a similar mechanism operates in the vascular response to ACh is uncertain. However, calcium-calmodulin can also stimulate HO2 activity by a mechanism that is independent and not additive with CK2 stimulation (5). Because TBB completely blocked the ACh response, calcium-calmodulin stimulation of HO2 by ACh is unlikely to play an important role in this vasodilatory response. In contrast, increased HO activity induced by glutamate in isolated piglet microvessels was blocked by a calmodulin inhibitor and not by TBB (17).

Previous work showed that SnPPIX inhibited the ACh response 2 days after polymeric Hb transfusion despite recovery explanation for dependence of the ACh response on HO activity because the ACh response could be blocked by SnPPIX within an hour after the Hb transfusion. Moreover, immunoblots of whole brain failed to detect an increase in HO1 activity 48 h after Hb transfusion when SnPPIX remained effective in inhibiting ACh-evoked dilation (28). The lack of effect of SnPPIX on the ACh response in HO2−/− mice after Hb transfusion indicates that HO1 activity was not being rapidly upregulated and contributing to the ACh response.

nonadrenergic, noncholinergic autonomic control of the intestines smooth muscle (35). In cerebral vascular smooth muscle, NO and CO may interact in a complex, nonadditive manner by directly acting on K+ channels to produce hyperpolarization (13, 14), by increasing cGMP to directly produce vasorelaxation, and by NO-induced increases in cGMP that enable CO-evoked dilation in an age-dependent manner (3, 12, 18).

The results in HO2−/− mice, which had a smaller dilatory response to ACh than HO2+/+ mice, also support a contribution of HO activity to ACh-evoked dilation of pial arterioles. However, the 14% attenuation in the HO2−/− mice was less than the 51% attenuation produced by SnPPIX in HO2+/+ mice. This difference in the magnitude of attenuation could be attributed to SnPPIX also acting on HO1 or other proteins in HO2+/+ mice, but this possibility is unlikely because SnPPIX had no effect on the ACh response in HO2−/− mice. More likely, HO2−/− mice have adapted to the chronic loss of HO2 by upregulating pathways that do not require CO.

Role of HO2 after Hb transfusion. The present findings in mice agree with earlier studies with ACh-induced dilation in cats and ADP-induced dilation in rats, which showed that endothelium-dependent dilators (which normally depend on NO) also become heavily dependent on HO activity after exchange-transfusion of polymeric Hb (28). Hence, this upregulation of the HO vascular signaling by Hb transfusion appears ubiquitous among different species and different endothelium-dependent agonists. Upregulation of HO activity is not attributable to a decrease in hematocrit and secondary decreases in endothelial shear stress because SnPPIX had no effect on the ACh response after exchange transfusion with an albumin solution that achieved similar hematocrit reductions (28). Thus this upregulation is likely related to properties of the Hb polymer per se. Possible factors to consider are release of free heme from a small fraction of the polymers, formation of reactive oxygen species by a small fraction of Hb that might be converted to highly reactive ferryl heme, or increased tissue oxygenation that leads to a shift in signaling pathways.

The release of heme could provide increased substrate for HO2 or induce HO1. However, induction of HO1 is not a likely
of plasma Hb to near-normal levels (28). This finding indicates that increased oxygenation afforded by this O₂ carrier is not required for the HO dependence of the ACh response. In addition, scavenging of endothelium-derived NO by plasma-based Hb does not fully account for the HO dependence of the ACh response because the ACh response is normal in cats after transfusion of either tetrameric cross-linked Hb or polymeric Hb and the ACh response is still completely blocked by L-NNA after Hb transfusion. In the present study, the ACh response was also intact after Hb transfusion in HO2−/− mice, and the response was fully inhibited by L-NNA. Therefore, even in the absence of CO derived from HO2, the Hb present in the plasma space does not scavenge sufficient NO to impair an endothelium-dependent vasodilator response. Moreover, the equivalent ACh response in Hb-transfused and nontransfused HO2−/− mice infers that plasma-based Hb does not scavenge all of the CO required for the dilatory response.

The decrement in the ACh response seen between the first and second ACh exposures in HO2−/− mice (Fig. 1) was not statistically significant in the HO2−/− mice transfused with Hb and treated with TBB during the second exposure (Fig. 4). It is unclear whether the lack of a time-dependent effect in the latter group is a true effect of genotype or Hb transfusion or a falsely negative effect of an underpowered sample size. In any case, this difference appears to be of marginal physiological significance.

Perspectives and Significance

Acellular Hb solutions are being tested for use in various clinical scenarios. One concern with the use of these solutions is that endothelial function might become impaired, especially if cell-free Hb scavenges more NO or possibly CO than that scavenged by Hb in red blood cells. Although the present work demonstrates intact cerebrovascular dilation to an endothelium-dependent agonist in healthy animals in the presence of Hb in the plasma, the signaling mechanism for endothelium-dependent dilation becomes more complex by relying more heavily on HO2 activity. Because cardiovascular disease can impair endothelial function, it will be important to determine whether Hb-based O₂ carriers adversely impact endothelial signaling and smooth muscle coupling in specific disease states.

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DISCLOSURES

E. Bucci and the University of Maryland are holders of a patent on the zero-link bovine hemoglobin polymer used in this study. R. C. Koehler and H. Kwansa were paid consultants to Oxyvita, Inc., holder of the licensing rights to the zero-link bovine hemoglobin polymer. The terms of this arrangement were managed by Johns Hopkins University in accordance with its conflict of interest policies.

REFERENCES


