Flow-Induced Dilation Is Mediated by Akt-Dependent Activation of Endothelial Nitric Oxide Synthase-Derived Hydrogen Peroxide in Mouse Cerebral Arteries

Annick Drouin, MSc; Eric Thorin, PhD

Background and Purpose—Endothelial nitric oxide synthase produces superoxide under physiological conditions leading to hydrogen peroxide (H$_2$O$_2$)-dependent dilations to acetylcholine in isolated mouse cerebral arteries. The purpose of this study was to investigate whether H$_2$O$_2$ was involved in flow-mediated dilation (FMD).

Methods—Cerebral arteries were isolated from 12-2-week-old C57Bl/6 male mice. FMD (0 to 10 $\mu$L/min, 2-$\mu$L step increase at constant internal pressure) was induced in vessels preconstricted with phenylephrine (30 $\mu$mol/L). Simultaneously to diameter acquisition, H$_2$O$_2$ or nitric oxide production was detected by the fluorescent dyes CMH$_2$CFDA or 4,5-diaminofluorescein diacetate, respectively. Results are expressed as mean±SEM of 6 to 8 mice.

Results—FMD (at 10 $\mu$L/min, 25±3% of maximal diameter) was prevented ($P<0.05$) by endothelium removal (6±1%) or endothelial nitric oxide synthase inhibition with N-nitro-L-arginine (11±1%) but not by the specific nitric oxide scavenger 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl3-oxide ($24±3%$). Addition of PEG-catalase and silver diethyl dithio-carbamate (superoxide dismutase inhibitor) reduced ($P<0.05$) FMD to 10±2% and 15±1%, respectively. Simultaneously to FMD, H$_2$O$_2$-associated rise in fluorescence ($+133±19$ a.u.) was prevented by N-nitro-L-arginine, PEG-catalase, and silver diethyl dithio-carbamate ($+55±10$, $+64±4$, and $+50±10$ a.u., respectively; $P<0.05$). Inhibition of FMD by PEG-catalase was fully restored by the addition of tetrahydrobiopterin, a cofactor of endothelial nitric oxide synthase (23±3%); this functional reversal in dilation was associated with the simultaneous increase in nitric oxide-associated fluorescence ($+418±58$ a.u., $P<0.05$), which was prevented by 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl3-oxide ($+93±26$ a.u.). Akt inhibition with triciribine prevented FMD and H$_2$O$_2$-associated rise in fluorescence (3±1% and $+23±4$% a.u., respectively; $P<0.05$), but not acetylcholine-induced dilation.

Conclusion—In healthy C57Bl/6 mouse cerebral arteries, Akt-dependent activation of endothelial nitric oxide synthase-derived H$_2$O$_2$ mediates flow-dependent dilation. (Stroke. 2009;40:1827-1833.)

Key Words: endothelium, nitric oxide, oxygen radicals, resistance arteries

S

cubin stress is one of the most important physiological stimuli for vasodilation and is greatly implicated in the regulation of vascular tone and vascular homeostasis by contributing to the maintenance of organ perfusion and vascular integrity.1,2 Several studies have evaluated the implication of endothelial nitric oxide synthase (eNOS) and cyclo-oxygenase (COX) derivatives in flow-mediated dilation (FMD).3–6 However, the implication of each endothelium-dependent relaxing factor (EDRF) varies among vascular beds and pathological states.3,7–11

The release of superoxide (O$_2^·$) has been reported during FMD both in peripheral12,13 and cerebral arteries.14,15 Although an increase in reactive oxygen species (ROS) is traditionally considered as a pathological response, recent investigations show that ROS are implicated in the regulation of vascular function.12,16–18 It is unclear, however, if this concept applies to the physiological regulation of vascular tone and FMD. In a recent study, our group showed that hydrogen peroxide (H$_2$O$_2$) is an EDRF produced by eNOS activity after muscarinic receptor stimulation.19 H$_2$O$_2$ was also reported to induce endothelium-dependent and -independent dilation in healthy rat cerebral arteries18 and identified as an EDRF in FMD of human coronary arteries.1 However, these results led to the hypothesis that H$_2$O$_2$ could be physiologically implicated in the regulation of cerebral arteries.

The origin of O$_2^·$ that leads to the physiological formation of H$_2$O$_2$ is still unsettled. eNOS, however, is able to generate O$_2^·$ during enzymatic cycling.20,21 The eNOS-dependent generation of O$_2^·$ is nonetheless proposed to be functionally significant only in pathological conditions and related to the limited availability of L-arginine, the substrate of eNOS as well as of its essential cofactor tetrahydrobiopterin (BH$_4$).22,23 In a recent study, Shimokawa’s group suggested that the

Received September 8, 2008; final revision received October 15, 2008; accepted October 29, 2008.

From the Université de Montréal, Department of Surgery, Institut de Cardiologie de Montréal, Montréal, Québec, Canada.

Correspondence to Eric Thorin, PhD, Institut de Cardiologie de Montréal, Centre de recherche, 5000, rue Bélanger, Montréal, Québec, H1T 1C8, Canada. E-mail eric.thorin@umontreal.ca

© 2009 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.108.536805

1827
NOs system has diverse vasodilator functions depending on the vessel size leading to superoxide production rather than nitric oxide in small vessels. There is therefore a need for determining the physiological role of eNOS derived H$_2$O$_2$ in FMD in cerebral arteries.

We hypothesized that eNOS produces physiologically relevant levels of free radicals leading to H$_2$O$_2$-dependent FMD in healthy mouse cerebral arteries.

Materials and Methods

Animals and Tissue Preparation

The procedures and protocols were performed in accordance with our institutional guidelines and the Guide for the Care and Use of Laboratory Animals of Canada. Experiments were conducted on cerebral arteries isolated from 3-month-old male C57Bl/6 mice (29±1 g; n=66; Charles River Laboratories, Quebec, Canada) using a method previously described. Mice were euthanized by CO$_2$ inhalation and the brain was rapidly removed and placed in ice-cold physiological salt solution (PSS) of the following composition (mmol/L): NaCl 130, KCl 4.7, CaCl$_2$ 1.6, MgSO$_4$ 1.17, NaHCO$_3$ 14.9, KH$_2$PO$_4$ 1.18, EDTA 0.026, glucose 10. In all PSS the PSS was oxygenated by a gas mixture containing 12% O$_2$, 5% CO$_2$, and 83% N$_2$, generating a PO$_2$ of 150±10 mm Hg. Cerebral arteries (anterior, posterior, and posterior communicating; internal diameter of 136±2 μm when pressurized at 60 mm Hg) were carefully isolated, cannulated at both ends, and pressurized at 60 mm Hg on a pressure myograph (Living Systems Instrumentation, Burlington, VT). The tips of the pipettes used in the study were calibrated and carefully checked to match the vessel inner diameter to minimize resistances.

Reactivity Studies

An equilibration period of 40 minutes was allowed before starting the experiment and the resulting myogenic tone was measured ([D$_{after}$−D$_{before}$/D$_{max}$]*100, where D$_{before}$ is the diameter before equilibration, D$_{max}$ is the diameter after equilibration time, and D$_{max}$ is the maximal diameter obtained in Ca$^{2+}$-free solution. Similar levels of preconstrictions with phenylephrine (PE; 10 to 30 μmol/L; Table) were obtained before each experiment. FMD was induced using a flow control peristaltic pump (Living Systems Instrumentation) directly connected to the pressured myograph. A single cumulative FMD curve (0 to 10 μL/min, 2-μL step increase at constant internal pressure) was performed on each segment. Two minutes were allowed for each 2-μL step increase. Shear stress was calculated using the following equation: $\tau = 4\pi Q/\pi r^2$ where $r$ is the shear stress, $Q$ is the flow rate through the lumen, and $r$ is the inside radius. In this study, the calculated shear stress was comparable to the physiological value of shear stress in arterioles of this size.

We used (1) PEG-catalase (50 and 100 U/mL); (2) N-nitro-L-arginine (L-NNA; 10 μmol/L), an eNOS inhibitor; (3) pyruvate (3 mmol/L), a H$_2$O$_2$ scavenger; (4) silver diethyldithiocarbamate (DETC; 1 mmol/L), a nitric oxide scavenger; (5) triciribine (1 μmol/L), an Akt protein inhibitor; (6) 1H-[1,2,4]-oxadiazole-4,3-dimethyloxazole-4,3-aminooxazol-1-oxide (ODQ; 10 μmol/L), a soluble guanylate cyclase inhibitor; (7) triciribine (1 μmol/L), an Akt protein inhibitor; (8) DT-3 (25 mmol/L), a cell-permeant 3G protein kinase inhibitor; (9) BH$_4$ (1 μmol/L); (11) apocynin (10 μmol/L), a NAD(P)H oxidase inhibitor; (12) L-arginine (5 mmol/L); and (13) detaNONOate (0.1 μmol/L), a nitric oxide donor. Acetylycholine (ACH; 30 μmol/L) and H$_2$O$_2$ (10 μmol/L) were also used to induce dilation. All drugs were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada), except for triciribine and DT-3, which were purchased from Calbiochem EMD Chemicals Inc (San Diego, Calif). All drugs were directly added to the bath chamber (extraluminal) and the final concentration of ethanol or DMSO never exceeded 0.1%.

Table. Myogenic Tone (MT), Contraction With PE (10 or 30 μmol/L), and Maximal Dilation to FMD (10 μL/min) of Cerebral Arteries Isolated From C57Bl/6 Male Mice*

<table>
<thead>
<tr>
<th>Groups</th>
<th>MT (%)</th>
<th>PE (%)</th>
<th>FMD (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control conditions</td>
<td>8±1</td>
<td>40±2</td>
<td>25±3</td>
<td>13</td>
</tr>
<tr>
<td>+L-NNA (10 μmol/L)</td>
<td>21±4†</td>
<td>46±5</td>
<td>11±1†</td>
<td>12</td>
</tr>
<tr>
<td>+PEG-catalase (50 U/mL)</td>
<td>15±3†</td>
<td>43±3</td>
<td>16±1†</td>
<td>12</td>
</tr>
<tr>
<td>+PEG-catalase (100 U/mL)</td>
<td>19±7†</td>
<td>46±3</td>
<td>10±2†</td>
<td>3</td>
</tr>
<tr>
<td>+BH$_4$ (1 mmol/L)</td>
<td>16±3†</td>
<td>42±4</td>
<td>28±3†</td>
<td>11</td>
</tr>
<tr>
<td>+l-arginine (5 μmol/L)</td>
<td>12±8</td>
<td>49±5</td>
<td>24±1†</td>
<td>6</td>
</tr>
<tr>
<td>+PTIO (100 μmol/L)</td>
<td>8±2</td>
<td>40±4</td>
<td>26±2†</td>
<td>8</td>
</tr>
<tr>
<td>+ODQ (1 μmol/L)</td>
<td>25±12†</td>
<td>49±4</td>
<td>4±2†</td>
<td>4</td>
</tr>
<tr>
<td>+DT-3 (25 mmol/L)</td>
<td>7±2</td>
<td>39±6</td>
<td>11±1†</td>
<td>3</td>
</tr>
<tr>
<td>+BH$_4$+PTIO</td>
<td>15±4†</td>
<td>39±7</td>
<td>20±4†</td>
<td>5</td>
</tr>
<tr>
<td>+BH$_4$+PEG-catalase</td>
<td>18±5†</td>
<td>45±5</td>
<td>26±3†</td>
<td>6</td>
</tr>
<tr>
<td>+BH$_4$+ODQ</td>
<td>12±5</td>
<td>45±4</td>
<td>7±2†</td>
<td>5</td>
</tr>
<tr>
<td>+L-NNA+indomethacin (10 μmol/L)</td>
<td>8±3</td>
<td>36±3</td>
<td>10±3†</td>
<td>5</td>
</tr>
<tr>
<td>+PEG-catalase+PTIO</td>
<td>7±2</td>
<td>39±4</td>
<td>14±3†</td>
<td>4</td>
</tr>
<tr>
<td>+Triciribine (1 μmol/L)</td>
<td>11±3</td>
<td>45±5</td>
<td>3±1†</td>
<td>4</td>
</tr>
<tr>
<td>+Apocynin (10 μmol/L)</td>
<td>12±5</td>
<td>45±4</td>
<td>33±4</td>
<td>4</td>
</tr>
<tr>
<td>-Endothelium</td>
<td>12±5</td>
<td>39±9</td>
<td>6±1†</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean±SEM. *MT, PE, and FMD are expressed as the percentage of the maximal diameter. †P<0.05 compared with control conditions.

Fluorescence Studies

Pressurized cerebral arteries were incubated in oxygenated PSS (37°C) containing either 5 μmol/L of 5- and 6-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (DCF-DA, a ROS-reacting fluorescent dye; Molecular Probe, Invitrogen, Burlington, Ontario, Canada) or 10 μmol/L of 4,5-diaminofluorescein diacetate (DAF-2, a nitric oxide-reacting fluorescent dye; Calbiochem, San Diego, Calif) 30 minutes before the beginning of the experiment, with or without inhibitors. Vessels were then washed with PSS, preconstricted with PE, and dilated with flow (0 to 10 μL/min) or with a single dose of ACh (30 μmol/L) while recording simultaneously the changes in diameter and in fluorescence intensities of fluorescein retained intracellularly after cleavage of the acetate moieties. Fluorescence intensities at 492 to 495 nm (excitation) were measured at 520 nm with an IonOptix Acquire system (IonOptix, Milton, Mass). Before each experiment, basal fluorescence intensity was recorded. Results represent differences between stimulated and basal intensity.

Statistics

$n$ refers to the number of animals used in each protocol. Continuous variables are expressed as mean±SEM. The maximal diameter (D$_{max}$) was determined by changing the PSS to a Ca$^{2+}$-free PSS. Dilations are expressed with the following equation: ([D$_{after}$−D$_{min}$/D$_{max}$−D$_{min}$])*100, where D$_{after}$ is the diameter at each flow value and D$_{min}$ is the diameter of PE-induced constriction. One-way analysis of variance were performed to compare the effect of the different inhibitors on FMD curves and the increase in fluorescence intensity at a flow rate of 10 μL/min. Differences were considered to be statistically significant when the probability value was <0.05 (Scheffe’s F test).
Results

Implication of Hydrogen Peroxide and Endothelial Nitric Oxide Synthase in Flow-Mediated Dilation

Flow-mediated dilations of cerebral arteries were reduced by nitric oxide synthase inhibition with L-NNA (10 μmol/L; Figure 1A; Table). The nitric oxide scavenger PTIO (100 μmol/L), however, had no impact on the dilation induced by flow (Figure 1A; Table). The cell-permeable PEG-catalase (50 and 100 U/mL) reduced FMD in a dose-dependent manner (Figure 1; Table). The likely involvement of H₂O₂ was further confirmed by the inhibitory effect of pyruvate (3 mmol/L), a H₂O₂ scavenger, and DETC (1 mmol/L), a superoxide dismutase inhibitor (Figure 1B; Table).

To confirm these pharmacological data, H₂O₂ production was assessed in pressurized vessels after incorporation of the fluorescent ROS-reactive dye, DCF-DA.¹⁹,²⁹,³⁰ FMD was associated with an increase in fluorescence intensity: ROS-dependent signals (Figure 2A, C) were abolished by L-NNA, PEG-catalase, DETC, and pyruvate, demonstrating the specificity of the dye for H₂O₂ in our experimental conditions. Apocynin, a NAD(P)H oxidase inhibitor, neither reduced FMD (Table) nor DCF-DA fluorescence (Figure 2C).

Nitric oxide production was assessed after incorporation of the fluorescent nitric oxide-reactive dye, DAF-2.¹⁹ FMD was not associated with an increase in fluorescence intensity, which was unaffected by the addition of PEG-catalase and DETC (Figure 2B, D).

In contrast, addition of the nitric oxide donor detaNONOate (0.1 μmol/L) induced a potent dilation of 73±5% of Dmax and increased DAF-associated fluorescence by 959±47 a.u.
Effects of Tetrahydrobiopterin on Endothelial Nitric Oxide Synthase Activity and Nitric Oxide Production

Addition of BH4 (1 mmol/L) did not alter FMD (Figure 3A; Table) but led to the production of nitric oxide as revealed by the appearance of a strong DAF-2-associated fluorescence that was prevented by PTIO and L-NNA (Figure 2B, D). Although PTIO or PEG-catalase alone did not alter FMD in the presence of BH4 (Table), the dilation to flow was prevented by combining PTIO and PEG-catalase (Table). This was associated with a reduction in both nitric oxide- and H2O2-associated fluorescence from 418±58 and 571±94 a.u. to 45±4 and 58±20 a.u., respectively (P<0.05).

In contrast to BH4, L-arginine (5 mmol/L) neither affected FMD (10 L/min; Table) nor nitric oxide-associated fluorescence (26±4 a.u.).

Vasodilator Mechanism of Action of Hydrogen Peroxide

ODQ (1 μmol/L), an inhibitor of the sGC, reduced the dilation triggered by flow as efficiently as endothelial denudation (Figures 3B and 4; Table). ODQ, however, did not prevent H2O2-associated rise in fluorescence (Figure 3C). In the presence of BH4, ODQ still reduced FMD (Figure 3A). Cell-permeant G protein kinase inhibition with DT-3 reduced FMD, but not the H2O2-associated rise in fluorescence (Figure 3B, C).

Endothelial Regulation of Flow-Mediated Dilation

FMD was strongly reduced by L-NNA with no additive effects of indomethacin, suggesting no involvement of COX derivatives during FMD of mouse cerebral arteries (Figure 4). FMD and its associated increase in H2O2 fluorescence were abolished by Akt inhibition with triciribine (Figure 5A, C; Table). In contrast, triciribine only limited ACh-induced endothelium-dependent dilation (30 μmol/L) and its associated increase in H2O2 fluorescence (Figure 5B, D).

Impact of Endothelial Nitric Oxide Synthase Activity on Myogenic Tone

L-NNA, PEG-catalase, DETC, and ODQ increased myogenic tone, whereas Akt inhibition and PTIO had no effect (Table). The rise in myogenic tone induced by L-NNA was prevented by COX-1/2 inhibition, suggesting that addition of L-NNA reveals a COX-derived contracting factor (Table).

Exogenous Hydrogen Peroxide Dilation

Exogenous H2O2 (10 μmol/L, n=4) induced a large dilation (65±4%). Addition of L-NNA or BH4 did not affect this response (60±6% and 52±7%, respectively). Addition of ODQ, however, prevented H2O2-induced dilation (6±2%).
H$_2$O$_2$-associated fluorescence in cerebral arteries incubated in arteries. This hypothesis is supported by the absence of eNOS activity and is responsible for FMD in mouse cerebral arteries.14 In addition, Akt-dependent activation of eNOS is mandatory for FMD, unlike for ACh-induced dilation. These results support the concept that eNOS-derived H$_2$O$_2$ is a physiologically relevant signaling molecule in healthy mice.

Endothelium removal abolished FMD in cerebral artery consistent with previous reports.5,7,13,32 Inhibition of eNOS by L-NNA limited the dilation similarly to H$_2$O$_2$ scavengers, whereas PTIO, a nitric oxide scavenger, had no impact. These results strongly suggest that H$_2$O$_2$ originates from stimulated eNOS activity and is responsible for FMD in mouse cerebral arteries. This hypothesis is supported by the absence of H$_2$O$_2$-associated fluorescence in cerebral arteries incubated in the presence of L-NNA and PEG-catalase and by the demonstration that FMD is not associated with an increase in nitric oxide-associated fluorescence. These data are in line with recent work showing that H$_2$O$_2$ is an important signaling molecule in cerebral14 and peripheral13 arteries.

We19 and others1,34 previously reported that H$_2$O$_2$ is an EDRF derived from eNOS activity after Ach-induced dilation of mouse arteries. It is known that eNOS is able to generate O$_2^*$ during enzymatic cycling.20,21 The eNOS-dependent generation of O$_2^*$ is proposed, however, to be only functionally significant in pathological conditions and related to the limited availability of eNOS cofactors.22,23 In this study, the addition of BH$_4$, but not L-arginine, induced an increase in nitric oxide-associated fluorescence, but neither BH$_4$ nor L-arginine had significant effect on the extent of FMD. This increase in nitric oxide-associated fluorescence intensity was blocked by PTIO and L-NNA, confirming that eNOS produces nitric oxide in the presence of an excess of BH$_4$. The level of BH$_4$ is significantly lower in the brain than in other mouse organs as previously reported by Heales’ group35; this could be a reason for the preferential production of H$_2$O$_2$, but it remains to be demonstrated. The NAD(P)H oxidase is known to be a source of superoxide and H$_2$O$_2$ during endothelium-dependent dilation of rat cerebral arteries.14 Our data, those of Rosenblum,15 and of Sobey’s group14 demonstrate that free radicals are involved in the physiological regulation of the cerebrovascular tone. Free radicals, however, are commonly associated with endothelial dysfunction and cellular damages triggered by oxidative stress.34 Many physiological regulatory effects are mediated by H$_2$O$_2$ and other ROS that are chemically derived from superoxide.36 NAD(P)H, for example, is essential for the regeneration of GSH from GSSG, reactivation of H$_2$O$_2$-inactivated catalase, and regeneration of thioredoxin.37 It is therefore essential to maintain adequate levels of NAD(P)H as well as the normal function of these enzymes. Hence, controlled production of free radicals has a physiological meaning, whereas unregulated oxidative stress is deleterious.

The fact that PTIO addition blocked the rise in nitric oxide-associated fluorescence after the addition of detaNONOate demonstrates the specificity of DAF-2 dye for nitric oxide as previously discussed.19 DCF-DA is known to recognize all ROS species at high concentrations such as O$_2^*$, OH-, and H$_2$O$_2$ with a greater specificity for ONOO$^-$.38 DCF-DA fluorescence signal was, however, abolished by PEG-catalase, pyruvate, and DETC, but not PTIO (data not showed), demonstrating greater selectivity of the dye for H$_2$O$_2$ than nitric oxide or its derived reactive nitrogen species. Hence, in these healthy arteries, and in our experimental conditions, peroxynitrites are not produced.

The mechanism of eNOS activation by flow is not fully understood. It has been proposed that Akt-dependent phosphorylation of eNOS is essential for nitric oxide production.14 Our study demonstrates that FMD is abolished by Akt inhibition and this was associated with a reduction in H$_2$O$_2$-associated fluorescence. Thus, shear stress-dependent activation of Akt is responsible for eNOS activation. To support this hypothesis, we used another Akt inhibitor (LY294002). LY294002, however, is also a PI3-K inhibitor and reduced the precontraction induced by PE (data not shown), preventing its use. In contrast to flow, muscarinic receptor activation directly promotes eNOS activation without requiring Akt-
dependent activation. Importantly, neither the dilation nor 
H2O2-associated fluorescence was abolished by Akt inhibi-
tion after muscarinic receptor stimulation. Akt inhibition 
reduced, nonetheless, ACh-dependent dilation, suggesting 
that there is a basal level of Akt activity influencing eNOS 
cycling.

Addition of ODQ to block the sGC abrogated FMD 
without affecting H2O2-associated fluorescence. Further-
more, exogenous H2O2-induced dilation was unaffected 
by L-NNA or BH4, but prevented by ODQ, which is in agree-
ment with our previous report.19 To further validate the 
involvement of the cGMP pathway in FMD, we used a 
cell-permeant G protein kinase inhibitor. FMD dilation, but 
not H2O2 production, was abolished by DT-3, confirming 
the implication of cGMP in H2O2-dependent FMD. In the pre-

cence of BH4, FMD was also abolished by ODQ, confirming 
that H2O2 shares with nitric oxide a similar vasodilator 
pathway.18,19

FMD was not completely blocked by L-NNA with no 
additive effects of indomethacin, suggesting the involvement of 
another EDRF. Previous studies1,7 suggested the role of an 
EDRF during FMD. This factor may account for the residual 
response obtained in the presence of L-NNA. It is also 
interesting that COX inhibition had no effect on FMD. This is 
another difference with ACh-induced endothelium dependent 
dilation of cerebral arteries in which prostacyclin signifi-
cantly contributes to this response.19 Like in our previous 
report,19 myogenic tone was increased by L-NNA but reduced 
by combining indomethacin, suggesting that in the absence 
of nitric oxide, a COX-derived contracting factor is produced or 
its effect revealed. We observed the same response in renal 
arteries,40 which suggests that both constrictors and dilators 
coexist to regulate tone. In age and in the presence of risk 
factor for cardiovascular diseases, the expression of these 
contracting factors dominate as a consequence of a reduced 
nitric oxide synthase activity.31–43

In conclusion, our results suggest that flow triggers Akt-
dependent eNOS activation leading to a dilation essentially 
induced by H2O2 in pressurized cerebral arteries isolated 
from healthy and young C57Bl/6 mice. Like nitric oxide, 
H2O2 activates the sGC. Our results highlight the multifac-
eted function of eNOS in its mechanisms of regulation of 
vascular function.

Sources of Funding
This work has been supported in part by the Foundation of 
Montreal Heart Institute, the Heart and Stroke Foundation of 
Quebec, and the Canadian Institute for Health Research (MOP87388). A.D. holds the 
Frederick Banting and Charles Best Canada Graduate Scholarships–
Doctoral Award in association with the Canadian Institute for Health Research.

Disclosures
None.

References
1. Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Guttermann DD. Role for 
hydrogen peroxide in flow-induced dilation of human coronary arterioles. 
2. Phillips SA, Hatoum OA, Guttermann DD. The mechanism of flow-
duced dilation in human adipose arterioles involves hydrogen peroxide 
Lüscher TF. Nitric oxide is responsible for flow-dependent dilation of 
human peripheral conduit arteries in vivo. Circulation. 1995;91: 
1314–1319.
4. Koller A, Sun D, Huang A, Kaley G. Corelease of nitric oxide and 
prostaglandins mediates flow-dependent dilation of rat gracilis muscle 
5. Shipley RD, Kim SJ, Muller-Delp JM. Time course of flow-induced 
vasodilation in skeletal muscle: contributions of dilator and constrictor 
6. Fuji K, Heistad DD, Faraci FM. Flow-mediated dilation of the basilar 
Guttermann DD. Flow-induced dilation of human coronary arterioles: 
important role of Ca(2+)-activated K(+) channels. Circulation. 2001; 
8. Kuo L, Chilian WM, Davis MJ. Interaction of pressure- and flow-
duced responses in porcine coronary resistance vessels. Am J Physiol. 1991;261: 
H1706–H1715.
9. Koller A, Sun D, Kaley G. Role of shear stress and endothelial prosta-
glandins in flow- and viscosity-induced dilation of arterioles in vitro. Circ 
10. Muller-Delp JM, Spier SA, Ramsey MW, Delp MD. Aging impairs 
dependent vasodilation in rat skeletal muscle arterioles. Am J 
contributes to the maintenance of flow-induced dilation in arterioles of 
12. Laurindo FR, Pedro Mde A, Barbeiro HV, Pileggi F, Carvalho MH, 
Augusto O, da Luz P. Vascular free radical release. Ex vivo and in vivo 
evidence for a flow-dependent endothelial mechanism. Circ Res. 1994; 
74:769–770.
Mitochondrial sources of H2O2 generation play a key role in flow-
dilated-mediated dilation in human coronary resistance arteries. Circ 
14. Paravincini TM, Miller AA, Drummond GR, Sobej CG. Flow-induced 
cerebral vasodilation in vivo involves activation of phosphatidylinositol-3 
kinase, NADPH-oxidase, and nitric oxide synthase. J Cerebr Blood Flow 
Metab. 2006;26:836.
15. Rosenblum WI. Hydroxyl radical mediates the endothelium-dependent 
relaxation produced by bradykinin in mouse cerebral arterioles. 
16. Rush JW, Ford RJ. Nitric oxide, oxidative stress and vascular endothel-
ium in health and hypertension. Clin Hemorheol Microcirc. 2007;37: 
185–192.
17. Ungvari Z, Wolin MS, Csiszar A. Mechanosensitive production of 
reactive oxygen species in endothelial and smooth muscle cells: role in 
18. Sobej CG, Heistad DD, Faraci FM. Mechanisms of bradykinin-induced 
19. Drouin A, Thorin-Trescases N, Hamel E, Falck JR, Thorin E. Endothelial 
nitric oxide synthase activation leads to dilatory H2O2 production in 
20. Phoroomchathara S, Tsai P, Rosen GM. The generation of free radicals by 
EE. Origin of superoxide production by endothelial nitric oxide synthase. 
22. Alp NJ, Chanon KM. Regulation of endothelial nitric oxide synthase by 
23. Katusic ZS, d’Uscio LV. Tetrahydrobiopterin. Mediator of endothelial 
24. Takagi A, Morikawa K, Tsutsui M, Morayama Y, Tereus E, Yamagishi H, 
Ohashi J, Yada T, Yanagihara N, Shimokawa H. Crucial role of nitric 
oxide synthases system in endothelium-dependent hyperpolarization in 
Assessment of vascular wall shear stress in implications for athero-


Flow-Induced Dilation Is Mediated by Akt-Dependent Activation of Endothelial Nitric Oxide Synthase-Derived Hydrogen Peroxide in Mouse Cerebral Arteries
Annick Drouin and Eric Thorin

Stroke. 2009;40:1827-1833; originally published online March 12, 2009;
doi: 10.1161/STROKEAHA.108.536805
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/40/5/1827