Modulation of Basal and Postischemic Leukocyte-Endothelial Adherence by Nitric Oxide

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Background and Purpose—Recent studies indicate that leukocytes are important contributors to secondary vascular and parenchymal injury after cerebral ischemia. The present study was undertaken to define nitric oxide (NO)—based mechanisms that regulate leukocyte-endothelial interactions in the cerebral vasculature, how these mechanisms are affected by cerebral ischemia, and whether NO-based therapies can affect postischemic leukocyte dynamics.

Methods—Leukocyte adherence to pial venules of anesthetized newborn piglets was quantified by in situ fluorescence videomicroscopy through closed cranial windows during basal conditions and during reperfusion after 9 minutes of asphyxia. Nitric oxide synthase (NOS) was inhibited by local window superfusion of L-nitroarginine; superfusion of sodium nitroprusside was used to donate NO.

Results—Local inhibition of NOS under resting conditions increased leukocyte-endothelial adherence 2.2-fold and 3.9-fold over baseline values after 1 hour and 2 hours, respectively; this response was completely blocked by cosuperfusion with L-arginine. Cosuperfusion of superoxide dismutase reversed L-nitroarginine-induced leukocyte adherence by 89% and 63% at these respective time points. The extent of acute leukocyte adherence elicited by NOS inhibition was similar in magnitude to that observed during the initial 2 hours of reperfusion after asphyxia. Leukocyte adherence was not additionally increased in asphyxic animals treated with L-nitroarginine. Sodium nitroprusside robustly inhibited asphyxia-induced leukocyte adherence back to control levels.

Conclusions—NO exerts a tonic antiadherent effect in the cerebral microcirculation by inactivation of adherence-promoting superoxide radical formation. Cerebral ischemia is associated with an inhibition of NOS or lower levels of NO, which results in leukocyte-endothelial adherence that can be prevented by NO donors. The latter may be useful therapeutically to prevent the purported vascular and parenchymal dysfunction and injury caused by activated leukocytes in ischemic brain. (Stroke. 1998;29:1423-1430.)

Key Words: cerebral ischemia, global ▪ leukocytes ▪ nitric oxide ▪ reperfusion injury ▪ pigs

It is now recognized that the abnormal behavior of leukocytes accompanies many disease states. Accumulating evidence indicates that both focal and global cerebral ischemia can elicit an acute inflammatory response characterized, in part, by leukocytes adhering to microvessel endothelial cells, plugging capillaries, and extravasating into brain parenchyma.1–2 On reperfusion, this multistep response progresses from an early stage of coactivation of circulating leukocytes and cerebral endothelial cells, to expression of their respective adhesion molecules, to rolling and sticking of leukocytes to endothelial cells, and, within hours to days after the initial insult, to diapedesis of leukocytes from the intravascular to the extravascular space. Although not all studies support an injurious role for leukocytes in ischemic brain injury,3,4 the documented rheological and hemodynamic effects of adherent leukocytes in cerebral vessels and the potent destructive capability of the free radicals and proteases these cells contain5,6 strongly suggest that the inadvertent activation of this inflammatory cascade could be an important contrib-
paratively little is known about the control of leukocyte adherence by NO in the cerebral circulation, and results of the sole report to date 12 run counter to the prevailing consensus about NO-based regulation of leukocyte-endothelial interactions in noncerebral tissues. Thus, we undertook the present study to begin to elucidate mechanisms controlling NO-regulated leukocyte-endothelial interactions in the cerebral circulation.

**Materials and Methods**

**Animal Preparation and Drug Superfusion**

Fifty-six newborn piglets (age, 2 to 5 days) weighing 1.5 to 3.5 kg were used in experimental protocols that were consistent with Public Health Service guidelines and were approved by our institutional animal studies committee. The preparation for in vivo monitoring of leukocyte dynamics has been detailed previously.13 In brief, after a tracheostomy under ketamine hydrochloride anesthesia (20 mg/kg IM), animals were ventilated with a mix of room air and oxygen, and anesthesia was maintained for the remainder of the experiment with isoflurane (1.0% to 1.5%). End-tidal CO2 and transcutaneous O2 tension of paralytic agent (pancuronium 0.25 mg/kg per hour) and fluids (5% dextrose in 0.45% saline; 6 mL/kg per hour) and in the tracheostomy under ketamine hydrochloride anesthesia (20 mg/kg IM), animals were ventilated with a mix of room air and oxygen, and anesthesia was maintained for the remainder of the experiment with isoflurane (1.0% to 1.5%). End-tidal CO2 and transcutaneous O2 tension of paralytic agent (pancuronium 0.25 mg/kg per hour) and fluids (5% dextrose in 0.45% saline; 6 mL/kg per hour) and in the femoral artery for continuous recording of mean arterial blood pressure. Intermittent samples of arterial blood were obtained for measurements of gas tensions, glucose concentration, pH, and hematocrit. A thermoregulated heating pad and overhead heating lamp were used to maintain core body temperature at 38°C to 39°C, which we found in pilot studies to differ insignificantly from simultaneously measured cortical temperature, except during the last few minutes of the 9-minute asphyxic period (when cortical temperature could drop 1°C to 2°C, particularly if the animals arrested for more than 1 minute) and during the initial 15 minutes of reperfusion (at which time cortical temperature had returned to baseline after falling no more than 2°C).

After an 18-mm craniotomy and removal of the dura, a closed cranial window made of Plexiglas was mounted over the right parietal cortex. Through ports at the edge of the window, intracranial pressure was continuously monitored; juxtaposed ports were used to superfuse drug solutions made up in artificial CSF, as described previously.13 Buffer or drug solutions were introduced into the window space by superfusion at 1 mL/min for 1 minute, followed by a continuous superfusion rate of 50 µL/min for 2.0 or 2.5 hours, with the use of an automated syringe pump.

**Leukocyte Quantification by In Situ Fluorescence Videomicroscopy**

Leukocytes were fluorescently labeled in situ with rhodamine 6G, which stains 100% of circulating leukocytes as assessed by flow cytometry.13 The loading dose consisted of 2 mL/kg of a filtered 0.006-mg/mL solution administered intravenously over 5 minutes, 30 minutes before the first baseline imaging period commenced. One to 2 minutes before each 60-second imaging period, rhodamine 6G was infused at 800 µL/min to enhance labeling. Leukocyte dynamics in pial venules were recorded to videotape in real time with the use of a Newvicon tube camera mounted on an epifluorescence microscope, as described in detail previously.13 During off-line playback of the video recording, leukocyte adherence to the endothelium of the pial venular wall was quantified manually by counting the number of leukocytes adherent to the vessel within a defined venular network that included several secondary and tertiary (20 to 45 µm diameter) postcapillary branches and one or two larger venules (60 to 90 µm diameter) into which they drained. Adherence values reported indicate the number of leukocytes per square millimeter of total endothelial vessel surface examined as determined by image analysis software (two-dimensional surface area times π). As in a previous intravital study of cerebrovascular leukocyte adherence,12 we operationally defined adherent leukocytes as those remaining stationary within the venule for longer than 10 consecutive seconds; in pilot studies, we found adherence typically lasted at least 1 minute and was almost always “permanent” (>5 minutes) during extended periods of continuous observation.

**Protocols**

Two baseline imaging periods, at a 30-minute interval, were obtained in all animals after a 45-minute postsurgery stabilization period. At that time, windows were superfused for 2 hours with drug or animals were rendered asphyxic for 9 minutes by turning off the ventilator and clamping the respiratory tubing, and they were observed for 2 hours of reperfusion. Drug superfusion in asphyxic animals was initiated either 0.5 hour before asphyxia or at the start of reperfusion. At the end of the 9-minute asphyxic period, animals were hypotensive, hypoxic, bradycardic, and acidotic; these changes became normalized to fall within typical physiological ranges by 0.5 to 1.0 hour of reperfusion.13

Animals were randomly divided as follows: group 1 (n=13) served as a normoxic control group; in 5 of these animals, artificial CSF was superfused through the window for 2 hours after baseline measurements were obtained, but in the remaining 8 animals, buffer was not superfused. Because no significant differences in leukocyte adherence were noted between these groups at any time point (data not shown), these groups were combined into a single control group for later statistical comparison with other animal groups (with or without window superfusion). In group 2 (n=7), L-NA (100 µmol/L), an NOS inhibitor, was superfused for 2 hours after baseline measurements. In piglets, window superfusion of L-NA at this concentration inhibits cortical NOS more than 90%.16 Group 3 animals (n=5) were concomitantly superfused for 2 hours with L-NA (100 µmol/L) and L-arginine (10 mmol/L). Group 4 animals (n=6) were superfused for 2 hours with a mix of both L-NA (100 µmol/L) and SOD (60 U/mL; Oxis Pharmaceuticals). Group 5 animals (n=9) were rendered asphyxic, and artificial CSF buffer without drugs was superfused through the window space starting 0.5 hour before asphyxia at a rate equal to that used in the other groups. In group 6 animals (n=6), superfusion of L-NA was initiated 0.5 hour before asphyxia and continued throughout 2 hours of postasphyxic reperfusion. In group 7 animals (n=6), SNP (40 µmol/L) was superfused immediately on reperfusion after asphyxia; SNP solutions were protected from light throughout the experiment with foil wrapping. Video images were obtained in all animal groups at 1 and 2 hours of drug superfusion or postasphyxic reperfusion for quantification of adherent leukocytes. Pial arteriolar diameters were measured at the same times in the control group (group 1) and in nonasphyxic animals superfused with L-NA (group 2). These changes were compared with changes in pial arteriolar diameters observed in a final animal group (n=4) that was superfused with 40 µmol/L SNP for 2 hours during resting, nonasphyxic conditions.

**Statistical Analyses**

Differences in the physiological, hemodynamic, and leukocyte adherence parameters within and between groups were assessed by repeated-measures ANOVA with, respectively, Duncan’s or Dunnnett’s multiple-range test. P<0.05 was considered significant.

**Results**

There were no significant differences in the recorded physiological and hemodynamic values between animal groups.
Effects of local NOS inhibition with L-NA and asphyxia/reperfusion on leukocyte adherence in piglet pial venules, as measured by in situ fluorescence videomicroscopy. The increases in the actual number of adherent leukocytes per square millimeter of pial venular area are shown, relative to the number of leukocytes adherent to the venular area before drug administration or asphyxia. Superfusion of L-NA (100 μmol/L; ▲; n=7) through the cranial window for 2 hours resulted in leukocyte adherence similar to that observed during the initial 2 hours of reperfusion after asphyxia (●; n=9); both conditions resulted in leukocyte adherence significantly greater than that measured in untreated controls (●; n=13). The adherence-promoting effect of L-NA was reversed by cosuperfusion with a 100-fold molar excess of L-arginine (○; n=6). P<0.05 vs control group at the same time point.

During baseline conditions and during 2 hours of reperfusion after asphyxia. Similarly, no significant differences in maximum or minimum venular diameters, the areas of venular network measured, or baseline arteriolar diameters (32 to 43 μm) were noted between groups. No changes in systemic physiological variables occurred in response to local superfusion of the drugs indicated. Finally, there were no significant differences between groups with respect to the mean number of leukocytes adherent to cerebral venules under baseline conditions (39 to 87/mm²).

In nonischemic control animals (group 1), a slight increase in leukocyte adherence occurred over the 2-hour observation period relative to that measured during baseline conditions (Figure 1). At 1 hour and 2 hours of observation, adherence increased 21±7% and 39±11% above baseline, respectively; only the latter change was significantly greater than baseline values. Leukocyte adherence was significantly increased in animals in which cranial windows were continuously superfused with the NOS inhibitor L-NA (group 2); the increase in adherence was progressive over time, such that adherence at 2 hours of superfusion (163±65% above baseline) was significantly greater than that measured at 1 hour of reperfusion (49±29% above baseline). This L-NA-induced adherence was almost totally reversed by cosuperfusion of a 100-fold molar excess of L-arginine (group 3; Figure 1). Figure 1 also shows that the magnitude and temporal pattern of leukocyte adherence that was elicited by local NOS inhibition was nearly identical to that observed in animals subjected to asphyxia and 2 hours of reperfusion (group 5). When cortical NOS was inhibited in animals rendered asphyxic (group 6), no further increase in leukocyte adherence was observed at any time point relative to animals subjected to asphyxia alone (data not shown).

To begin to address the mechanism of leukocyte adherence after NOS inhibition, we tested the hypothesis that increases in superoxide free radical levels resulting from a loss of NO promoted such adherence. We reasoned that concomitant superfusion of SOD with L-NA would eliminate the increase in leukocyte adherence we witnessed with L-NA alone. Indeed, in these animals (group 4), leukocyte adherence was dramatically attenuated relative to group 2 L-NA-treated animals without SOD (Figure 2). In particular, leukocyte adherence after 1 hour of drug exposure was reduced to levels equivalent to those in untreated controls; by 2 hours of drug exposure, adherence was still significantly reduced relative to animals receiving L-NA alone but also became significantly greater than adherence levels observed in untreated controls at the same time point.

To examine the corollary hypothesis that exogenous NO could attenuate ischemia-induced increases in leukocyte adherence, the NO donor SNP was superfused through the cranial window of asphyxic animals at the initiation of reperfusion (group 7). This treatment resulted in a robust and significant reduction in asphyxia-induced leukocyte adherence to levels not significantly different from those in nonasphyxic, untreated controls (Figure 3).

Changes in pial arteriolar diameter in the control group and in animals superfused with either L-NA or SNP are shown in the Table. No significant change in arteriolar diameter was measured in control animals (group 1) over time. Superfusion of L-NA in group 2 animals did not significantly affect pial arteriolar diameter at any time point. Conversely, superfusion...
of SNP through the cranial window in a separate group of four animals resulted in a significant and steady dilation (61%) of pial arterioles at 1 and 2 hours of superfusion.

Discussion

The present study provides the first evidence in the cerebral circulation that (1) inhibition of NOS during nonischemic resting conditions results in an acute, arginine-reversible adherence of leukocytes to cerebral venules; (2) superoxide radicals are involved in mediating leukocyte adherence after NOS inhibition; (3) inhibition of NOS during ischemia and reperfusion does not exacerbate the extent of leukocyte adherence induced by ischemia/reperfusion alone; and (4) local administration of an NO donor can dramatically attenuate leukocyte adherence after cerebral ischemia. Thus, two lines of complementary evidence gathered herein support an important role for NO in modulating leukocyte-endothelial interactions in the cerebral circulation under both physiological and pathophysiological conditions. Our findings suggest that a balance between the antiadherent effects of NO and the proadherent effects of superoxide radical underlies the changes in leukocyte dynamics we observed with both NOS inhibition and ischemia/reperfusion. These findings are likely to have important implications for anti-inflammatory stroke therapy during the initial hours of reperfusion.

Endothelial cells, leukocytes, and platelets contain both constitutive and inducible NOS isoforms. In addition to influencing tissue perfusion, accumulating evidence gathered from studies of noncerebral vascular beds indicates that NO produced at the blood-endothelial interface also modulates leukocyte adherence, platelet aggregation, and endothelial permeability in a tonic fashion. In resting peripheral microcirculatory beds, for example, leukocytes adhere to venular endothelium after administration of NOS inhibitors. Under similar nonischemic conditions, we confirmed that local inhibition of NOS with L-NA elicited a progressive increase in leukocyte adherence to the pial venular microcirculation over 2 hours of continuous drug presentation and observation. As expected, this effect was arginine reversible. Given the well-established concept that, in most species, NO contributes a tonic dilative effect in the cerebral circulation secondary to its ongoing production by endothelial NOS, our findings indicate that tonically released NO also acts to inhibit the adherence of circulating leukocytes to cerebrovascular endothelium. Our results differ importantly from those in a recent cranial window study in rats, wherein topical L-NA administration (1 mmol/L) did not significantly elevate leukocyte adhesion. However, in the latter study, when the basal level of activation of circulating leukocytes and/or cerebrovascular endothelium was increased mildly with leukotriene B4 superfusion, subsequent L-NA administration promoted significant leukocyte adherence. It is difficult to identify underlying reasons for these discordant observations, given that the magnitude of trauma-induced histamine release, cytokine release, or mast cell degranulation in response to surgical preparation of a cranial window in rats and piglets is probably similar, but species-, anesthesia-, and age-dependent differences in receptor sensitivity, in the regulation of adhesion molecule expression, or in other unidentified parameters could be important. Clearly more studies of the control of leukocyte-endothelial adherence by NO in the cerebral circulation are warranted in other stroke models to resolve these important issues.

The mechanisms whereby tonically produced NO serves to inhibit leukocyte-endothelial interactions are likely to be multifactorial. Changes in vessel shear rate, interactions with superoxide radical, and alterations in adhesion molecule expression are likely candidates. For example, the vessel shear rate resulting from the tonic vasodilative effect of NO would be reduced after NOS inhibition, and leukocyte adherence might then be promoted secondary to a decrease in blood flow. However, the lack of change in pial arteriolar...
diameter in response to L-NA superfusion in piglets suggests that leukocyte adherence was invoked with little change in local cortical blood flow. This finding is consistent with the predominant dependence of the newborn piglet cerebral circulation on adenosine and prostanoids for the metabolic regulation of cerebral vascular resistance, with NO-based regulatory systems becoming operative only after maturation. 2\textsuperscript{3} Although vasoactive effects of this tonically produced NO are not realized (perhaps because of alterations in the sensitivity of downstream effector pathways in the vascular smooth muscle), our finding that leukocyte adherence was stimulated after NOS inhibition indicates that enough NO is produced at the blood-endothelial interface under baseline conditions in the piglet cerebral circulation to maintain an antiadhesive endothelial surface for circulating leukocytes independent of an effect on shear rate. NO exhibits a similar shear-independent effect on basal leukocyte adherence in the cat mesentery. 10

A primary mechanism whereby NO is likely to negatively affect leukocyte adherence is based on the avidity of NO for interacting with superoxide free radical. Endogenous NO competes with SOD to inactivate basally produced superoxide radical 2\textsuperscript{4,25}; thus, loss of NO after NOS inhibition could lead to increases in the levels of superoxide radical, a well-established proadherent molecule in a variety of microcirculatory beds. 2\textsuperscript{6,27} In fact, superfusion of NOS inhibitors increased oxidative stress–sensitive probe fluorescence in rat mesenteric venules before leukocyte adherence. 2\textsuperscript{0,22} indicative of increased radical production in response to loss of endogenous NO. Our finding that L-NA–induced adherence to cerebral venules is attenuated dramatically by concomitant superfusion of SOD supports the hypothesis that ongoing NO production balances ongoing oxidant formation and is consistent with similar findings resulting from coadministration of oxygen radical scavengers and NOS inhibitors in non cerebral tissues. 2\textsuperscript{0,28,28}

Both endothelial cells and leukocytes could serve as the cellular source of oxygen radicals in the face of declining NO levels during basal conditions. Mitochondrial-rich cerebral endothelial cells produce superoxide as a result of electron transport chain activity during aerobic metabolism, from cyclooxygenase-dependent formation of prostanoids, and from the activity of xanthine oxidase. 2\textsuperscript{8} Elegant intravital microscopy studies 2\textsuperscript{1} have demonstrated increases in hydroperoxide formation within endothelial cells in response to treatment with NOS inhibitors. In addition, considerable evidence is available documenting the ability of NOS inhibitors to increase free radical production by leukocytes. 2\textsuperscript{0} Conversely, NO can reduce free radical formation from activated neutrophils 2\textsuperscript{4,30} by inhibiting NADPH oxidase, 3\textsuperscript{1} the primary enzymatic source of leukocyte-derived free radicals. The prevention of L-NA–induced adherence with SOD that we and others documented 2\textsuperscript{6,28} suggests that superoxide radical may be the particular radical species responsible for promoting adherence, but similar antiadherent effects with catalase 2\textsuperscript{0} indicate that other downstream radical species (hydrogen peroxide and/or hydroxyl radical) may be involved as well.

In addition to influencing steady-state oxygen free radical levels, NO may regulate in a direct fashion the expression of endothelial and leukocyte adhesion molecules. There is evidence in the rat ilial mesenteric microcirculation 2\textsuperscript{9,32} that NO inhibits the endothelial, cyclic GMP–dependent expression of P-selectin, which in turn promotes rolling of leukocytes on the endothelium at sites of inflammation before their firm adherence. Such adherence is dependent on endothelial cell expression of intercellular adhesion molecule and vascular cell adhesion molecule, which also appear to be downregulated by NO. 3\textsuperscript{3,34} Although an NO-induced inhibition of the expression of the leukocyte CD18 adhesion molecule, the co-ligand for endothelial intercellular adhesion molecule, has been demonstrated in vitro with the use of cultured endothelium, 3\textsuperscript{5} similar support for this mechanism could not be demonstrated in vivo. 3\textsuperscript{6} Finally, NO may also tonically prevent leukocyte adherence in a more indirect way by inhibiting the production of proinflammatory chemoatractants.

There is now considerable evidence indicating that an acute inflammatory response occurs after cerebral ischemia, characterized by a progressive increase in leukocyte adherence and infiltration over the initial hours to days after the insult. 1 We demonstrated previously that the severe hypoxia and hypotension accompanying 9 minutes of asphyxia in piglets elicits significant leukocyte adherence even within the initial 2 hours of reperfusion. 13 The magnitude and time course of this adherence were nearly identical to those observed in the present study after L-NA superfusion. That no additional increase in leukocyte adherence occurred during the early posts ischemic reperfusion period after NOS inhibition by L-NA suggests that asphyxia-reperfusion resulted in a depletion of endogenous basal levels of NO. As found in other tissues, recent studies in brain 3\textsuperscript{7} document an attenuation or absence of NO-dependent vasoreactivity during the initial hours after ischemia, even though reactivity to NO donors remains intact, indicating that endothelial NOS function is impaired after ischemia and/or that NO is efficiently scavenged once it is produced. The robust increase in oxygen free radical formation occurring coincident with posts ischemic reperfusion 3\textsuperscript{8} is consistent with the latter possibility but does not explain how NO donors retain their vasoreactivity after ischemia. In either event, the data collectively suggest that a fall in basal NO levels after ischemia underlies, in part, the early leukocyte-endothelial adherence behavior we observed. Our studies only examined the initial 2 hours of reperfusion; thus, the time course over which a significant NO-based antiadherent effect might be reestablished, as well as the effect of large increases in NO production from inducible NOS on posts ischemic leukocyte adherence, remains undefined.

If the above hypothesis that NO exhibits multifunctional antiadherent activity is correct, then supplementing posts ischemic tissue with NO donors or NO precursors would be expected to attenuate the degree of leukocyte sticking after ischemia. Indeed, posts ischemic leukocyte adherence was dramatically reduced in our model when the organic nitrate NO donor SNP was superfused across the cortical surface at the start of reperfusion. Our findings in brain are consistent with results from intravital microscopy studies in the splanchn-
nic microcirculation wherein arginine supplementation or administration of a variety of NO donors decreases ischemia-induced leukocyte adherence, and endothelial dysfunction, and P-selectin expression in a similar fashion.

As alluded to above, direct quenching of superoxide radical is one mechanism whereby pharmacological augmentation of NO levels may attenuate leukocyte adherence after ischemia or after direct exposure to oxygen free radical—generating systems, since superoxide radicals potently stimulate adhesion molecule expression and leukocyte adherence. Indeed, superfusion of SOD attenuates ischemia-induced leukocyte adherence in our piglet model similar to SNP (J.M.G. et al., unpublished data, 1997). In addition to direct superoxide inactivation, NO may indirectly reduce superoxide formation as a result of its ability to suppress endothelial xanthine oxidase. This enzyme forms superoxide radical in cerebral endothelial cells when hypoxanthine and xanthine are converted to uric acid after ischemia-induced purine catabolism. There is also the possibility that an increase in blood flow accounts, in part, for the decrease in postsischemic leukocyte adherence after administration of NO donors. With the pial arteriolar dilation elicited by superfusion of SNP, we cannot rule out the possibility that the resultant increase in shear may have contributed to the SNP-induced reduction in leukocyte adherence; parallel control studies employing other common vasodilators like adenosine or prostacyclin are problematic given their NO dependence or their direct anti-adherent effects.

The role of NO in modulating postsischemic leukocyte adherence that we demonstrated herein may, in addition to its hemodynamic and platelet antiaggregatory effects, explain in part the findings that infarcts resulting from middle cerebral arterial occlusion are larger in endothelial NOS knockout mice, that early administration of NOS inhibitors exacerbates ischemic damage, and that l-arginine supplementation and NO donors decrease brain injury and improve outcome in a variety of stroke models. Our previous demonstration that blood-brain barrier breakdown in asphyxiated piglets results, in part, from adherent leukocytes suggests that reductions in postsischemic edema may also be realized with NO-based therapy secondary to reductions in postsischemic microvascular permeability; the latter has been documented in the rat mesenteric microcirculation.

In summary, we have demonstrated that NO inhibits leukocyte adherence to cerebral venules in a tonic fashion by inactivating basally produced superoxide radical, that the antiadherent effect of NO is lost during the initial hours of reperfusion after ischemia as a result of an impairment in NOS and/or an increase in free radical formation, and that NO supplementation can reverse ischemia-induced leukocyte adherence. Mechanistically, these effects of NO are much more complex than our end points indicate, and future work can elucidate how they are likely to vary depending on the nature of the ischemic insult, the relative extent of NO and oxygen free radical production, the status of many coexistent hemodynamic variables, and the time at which the inflammatory response to ischemia is examined.

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Editorial Comment

In 1993, Kubes and coworkers\(^1\) introduced the concept that NO functions as an important regulator of leukocyte adherence, possibly via roles in modulating local superoxide or hydroxyl radical levels and/or effects on expression of P-selectin or CD18. This role for NO in the tonic regulation of leukocyte adherence in the mesenteric circulation of the rat did not seem to operate in rat cerebral circulation, as reported by Lindauer et al\(^2\); thus, the antiadhesion effects of NO were thought to play a more limited role in brain versus microvascular beds outside of the central nervous system. However, Lindauer et al did report that NOS inhibitors augment leukocyte adherence in LT4-stimulated rat pial microcirculation. In the accompanying article by Gidday et al, we are shown that NO exerts tonic basal antiadherent effects in the pial microcirculation of newborn piglets and that loss of endogenous local NO production after asphyxia may play an important role in promoting the adherence of leukocytes. Thus, loss of NO could potentially magnify secondary damage produced by the acute inflammatory response. Factors related to species (rat versus piglet versus humans), age (mature versus immature), or model (focal or global ischemia versus asphyxial cardiopulmonary arrest) could potentially limit the importance of this observation. Nevertheless, it appears that another layer of complexity has been added to the potential roles for NO in the evolution or prevention of secondary damage in the injured brain—in this case, another putative favorable effect. This work brings to light a fundamental issue that deserves additional discussion.

Numerous recent studies in models of both ischemic and traumatic brain injury are demonstrating both detrimental and beneficial effects of an incredibly complex and highly interactive local inflammatory response. Some of the NO-mediated effects in this setting, related to the inducible form of NOS [iNOS], are considered part of traditional "inflammation." However, to be more encompassing, both NO and a variety of inflammatory participants seem to share this bipolar role in the injury response in brain, and we will take the liberty of discussing them together as part of a "tissue-injury response" rather than an "inflammatory response." Both detrimental and beneficial aspects of NO and many components of this tissue-injury response have been reported. This includes a variety of participants, such as cytokines, adhesion molecules, NF-\(\kappa\)B, and NO. Adding to the complexity, a variety of factors appear to determine whether beneficial or detrimental aspects dominate for any given component of this tissue-injury response. Included in this extensive list are factors such as the experimental model or specific clinical condition involved, the site of mediator production versus asphyxial cardiopulmonary arrest) could potentially be involved.
production (i.e., neuron, astrocyte, or microcirculation), the
timing of the event in question or of the therapeutic interven-
tion, and a variety of other contributors. Certainly for NO,
both beneficial and detrimental effects seem to be operating
in the injured brain. NO derived from neuronal NOS during
the excitotoxic response to ischemia/reperfusion may lead to
peroxynitrite formation, PARS activation, and neuronal
death.3,4 Under different redox conditions, however, NO-
mediated nitrosylation of the NMDA receptor or caspases
may attenuate neuronal death.5 Similarly, microcirculatory
effects of NO may help provide flow in the ischemic
penumbra and, as we are shown here by Gidday et al,
attenuate local inflammation in injured brain.

Despite a number of studies targeting selected aspects of
the tissue-injury response after ischemic or traumatic brain
injury, the only approaches to date that have paid dividends in
the clinic (although still somewhat controversial) are aug-
mentation of reperfusion with thrombolytics in stroke6 (i.e.,
good, old-fashioned plumbing) or the application of a broad-
spectrum therapy such as hypothermia in traumatic brain
injury.7 Is the inflammatory (or tissue-injury) response both
too cybernetic and too bipolar to yield a therapeutic approach
that will translate into a clinical breakthrough? Are there
purely deleterious aspects of this response to target with
inhibitors? Are there components that should be targeted and
enhanced because they are exclusively and/or powerfully
beneficial? Despite what appears to many to be an “old
story,” our knowledge of how NO and many other inflam-
matory participants contribute to injury or repair in the
tissue-injury response is still cryptic at best. It may be that a
much more complete understanding of these pathways is
needed before a breakthrough can be developed. Although a
breakthrough therapeutic agent may be—at this
moment—innocently staring us in the face, it is certainly
possible that better therapies targeting highly selective as-
pects of the tissue-injury response need to be developed.
Before we know the answers to these questions, it is becom-
ing apparent that for NO and many other inflammation-
related aspects of the tissue-injury response, we have yet to
begin to answer the grade school questions of “who, what,
when, where, why, and how.”

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