Nitric Oxide and the Cerebral Circulation

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**Background** Nitric oxide (NO) is a potent vasodilator that was initially described as the mediator of endothelium-dependent relaxation (endothelium-derived relaxing factor, EDRF). It is now known that NO is produced by a variety of other cell types.

**Summary of Review** Endothelium produces NO (EDRF) under basal conditions and in response to a variety of vasoactive stimuli in large cerebral arteries and the cerebral microcirculation. Endothelium-dependent relaxation is impaired in the presence of several pathophysiological conditions. This impairment may contribute to cerebral ischemia or stroke. Activation of glutamate receptors appears to be a major stimulus for production of NO by neurons. Neuronally derived NO may mediate local increases in cerebral blood flow during increases in cerebral metabolism. NO synthase-containing neurons also innervate large cerebral arteries and cerebral arterioles on the brain surface. Activation of parasympathetic fibers that innervate cerebral vessels produces NO-dependent increases in cerebral blood flow. Increases in cerebral blood flow during hypercapnia also appear to be dependent on production of NO. Astrocytes may release some NO constitutively, but astrocytes and microglia can release relatively large quantities of NO after induction of NO synthase in response to endotoxin or some cytokines. Expression of inducible NO synthase, perhaps in response to local production of cytokines, may exert cytotoxic effects in brain during or after ischemia.

**Conclusions** Because endothelium, neurons, and glia can all produce NO in response to some stimuli, the influence of NO on the cerebral circulation appears to be very important. Under normal conditions, constitutively produced NO influences basal cerebral vascular tone and mediates vascular responses to a diverse group of stimuli. The inducible form of NO synthase produces much greater amounts of NO that may be an important mediator of cytotoxicity in brain.

**Key Words** • cytotoxicity • endothelium-derived relaxing factor • microcirculation • nitric oxide

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**Furchgott and Zawadzki** first demonstrated that endothelium-derived relaxing factor (EDRF) can exert a major influence on vascular tone of the aorta in vitro. Subsequent studies indicate that production of EDRF influences tone in several vascular beds, including the cerebral circulation. The effects of NO may be particularly important in the cerebral circulation because it is now known that neurons and glia, in addition to endothelium, can produce NO.

This review will summarize recent concepts concerning (1) characteristics of NO synthase and its regulation; (2) production of NO by endothelium, neurons, and glia; (3) the role of NO as a mediator of cerebral vasodilation in response to stimuli such as hypercapnia and neuronal activation; and (4) the influence of NO during ischemia.

**Nitric Oxide Synthase**

Nitric oxide synthase converts L-arginine into NO and citrulline (Fig 1). Although it was initially described in endothelium, NO synthase activity has now been described in many cell types. Three distinct major forms of NO synthase have been cloned. Brain, endothelium, and macrophage isoforms appear to be products of different genes that have approximately 50% amino acid identity. NO synthase in brain and in endothelium have very similar properties, the major differences being that brain NO synthase is cytosolic and the endothelial enzyme is mainly a membrane-associated protein.

Functionally, NO synthase may be separated into constitutive and inducible forms. The constitutive form (present in brain and endothelium) may be active under basal conditions and can be further stimulated by increases in intracellular calcium that occur in response to receptor-mediated agonists or calcium ionophore (Fig 1). Constitutive NO synthase appears to be the "physiological" form of the enzyme and plays a role in a diverse group of biologic processes. In vitro studies suggest that the activity of brain NO synthase can be regulated in a negative feedback manner by NO itself. In the cerebral circulation, the primary target for constitutively produced NO is soluble guanylate cyclase located in vascular muscle (see below) (Fig 1).

In the presence of normal substrate, NO is made preferentially by NO synthase. However, in the absence of L-arginine, brain NO synthase can generate superoxide and hydrogen peroxide. This property of NO synthase has potential major implications for neurotoxicity and pathophysiological conditions such as ischemia.

In contrast to the constitutive form of the enzyme, an inducible, calcium-independent form was initially described in macrophages. It is now known that induction of NO synthase can occur in response to appropriate stimuli in many other cell types. This includes both cells that normally do not express a constitutive form of NO synthase, such as vascular smooth muscle...
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cerebral arterioles in response to acetylcholine may not
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vessels both in vitro (Fig 2) and in vivo.20-21 It is
synthase (NOS). NOS produces NO and L-citrulline from
arginine, and production of NO can be inhibited with certain
analogues of L-arginine, including N°-monomethyl L-arginine
(L-NMMA) and N°-nitro-L-arginine (L-NNA). NO diffuses freely to
target cells, where it activates guanylate cyclase, causing
increased production of cyclic GMP (cGMP).

Inducible NO synthase exhibits negligible activity
under basal conditions, but in response to factors such as
lipopolysaccharide and certain cytokines, expression
occurs over a period of hours. The induced form of the
enzyme produces much greater amounts of NO than the
constitutive form,2,4-11 and induced NO synthase appears
to be the "pathophysiological" form of the en-
zyme because high concentrations of NO can be toxic to
cells. Induction of NO synthase can be inhibited by
glucocorticoids and some cytokines.2 Relatively little is
known about posttranscriptional regulation of induced
NO synthase.2,11 Cytoxic effects of NO are probably
largely independent of guanylate cyclase and cyclic
GMP (cGMP) formation (Fig 1).11

Because NO has a very short half-life, quantification of
NO production is often very difficult. Therefore, many
studies of the biologic effects of NO have relied on studies
of the enzyme NO synthase and especially the use of
certain analogues of L-arginine that can be relatively
specific enzyme inhibitors. These inhibitors include N°,
monomethyl L-arginine (L-NMMA), N°-nitro-L-arginine
(L-NNA), and N°-nitro-L-arginine-methyl ester (L-
NAME) (Fig 1).2

Nitric Oxide From Endothelium

Constitutive Nitric Oxide Synthase

Immunocytochemical studies have localized NO syn-
thesis in endothelium, but not smooth muscle, of cerebral
arteries.17,18 In situ hybridization has demonstrated that
messenger RNA for a constitutive form of NO synthase is
present in cerebral endothelium.19

Nitric oxide is a potent dilator of cerebral blood
vessels both in vitro (Fig 2) and in vivo.20-21 It is
important to acknowledge that the EDRF released by
cerebral arterioles in response to acetylcholine may not
be NO per se but may be an NO-containing com-

compound.4,20,22-24 For simplicity, we will simply refer to the

relaxing factor produced by the various cell types in
brain as NO. After release by endothelium, NO stimu-
lates soluble guanylate cyclase in smooth muscle, result-
ing in a rise in cGMP and relaxation (Fig 1).1,13
Nitrovasodilators such as sodium nitroprusside and
nitrergic also increase formation of cGMP and
produce relaxation of cerebral vascular muscle (Fig 1).25-28 Methylen blue, which can inhibit soluble gua-
ylate cyclase under some conditions, attenuates relax-

ation of cerebral vessels in response to NO and
nitroprusside.27-29

Most studies suggest that L-arginine, the substrate for
formation of NO, has little effect on vascular tone of
cerebral blood vessels both in vitro and in vivo.30-37
These findings are consistent with the concept that
availability of L-arginine is not limiting for production of
NO under normal conditions. In contrast, in some in
vivo studies, topical application of L-arginine has been
observed to produce small to moderate dilator re-

sponses of cerebral arteries.38,39

The majority of evidence for endothelium-dependent
relaxation in the cerebral circulation has been obtained
using large cerebral arteries in vitro. Endothelium-
dependent relaxation in these vessels has been observed
in response to acetylcholine and a number of receptor-
mediated agonists in both animals and humans.34-40
Using inhibitors of NO synthase, endothelium-depen-
dent relaxation of large cerebral arteries to a diverse
group of agonists has subsequently been shown to be
dependent on formation of NO (Fig 3).34

Studies using endothelial damage, inhibitors of NO
synthase, a bioassay technique, and measurement of local
nitrite (an oxidation product of NO) release all suggest
that cerebral arterioles respond to acetylcholine through
an endothelium-dependent mechanism and release of
EDRF in vivo.34,56-59 In addition to acetylcholine, it is now
known that dilatation of the cerebral microcirculation in
response to serotonin, substance P, and ADP is also
dependent on formation of NO.32-34,37,38,40-43-66-66

Endothelium-dependent responses may also occur in
cerebral blood vessels in response to other cells. For
example, platelets and astrocytes produce endothelium-
dependent relaxation of cerebral arteries in vitro.67-68
The effect of aggregating platelets is probably mediated
by ADP, a potent endothelium-dependent vasodilator.67
Recent studies suggest that endothelium-dependent
relaxation of cerebral vessels also occurs in response to
astrocytes and is mediated by a lipoxygenase product
released constitutively by astrocytes in culture.68

![Diagram of NO synthesis and relaxation](image-url)
Several lines of evidence suggest that formation of NO occurs in cerebral blood vessels under basal conditions both in vitro and in vivo. In vitro, basal levels of cGMP are much greater in cerebral arteries with endothelium than in vessels without endothelium. Inhibitors of NO synthase decrease basal levels of cGMP and produce constriction of cerebral arteries in vitro that is endothelium dependent.

In vivo, local administration of inhibitors of NO synthase (L-NMMA, L-NNA, and L-NAME) produces constriction of cerebral blood vessels, and these same inhibitors decrease cerebral blood flow under basal conditions in several species. It is possible that a portion of the NO that influences basal tone in cerebral vessels is not of endothelial origin but is derived from glia or neurons.

In addition to exerting a tonic dilator influence on the cerebral circulation, basal release of NO may protect against oxygen radicals, which can destroy NO, impairs endothelial function in cerebral arterioles after cerebral ischemia, and alters responses of cerebral arterioles to substances such as norepinephrine and serotonin.

**Inducible Nitric Oxide Synthase**

The presence of an inducible form of NO synthase has also been described in cerebral blood vessels. Induction of NO synthase occurs in cerebral endothelium in response to inflammatory stimuli such as endotoxin and certain cytokines. Interferon gamma, alone but especially in combination with tumor necrosis factor, interleukin-1, or endotoxin, stimulates induction of NO synthase in cultured cerebral endothelium. A recent study suggests that induction of NO synthase also occurs in response to lipopolysaccharide in cerebral vascular muscle in vivo. This induction was associated with impaired responses of cerebral arteries to vasoconstrictor stimuli. Preliminary evidence suggests that lipopolysaccharide produces marked dilatation of cerebral arteries in vivo, which is dependent on formation of NO and may be mediated by induction of NO synthase.

**Pathophysiology**

Impaired endothelium-dependent relaxation of cerebral blood vessels has been observed during chronic hypertension, diabetes, hypercholesterolemia, subarachnoid hemorrhage, ischemia, and aging. In all cases, this impairment appears to be specific for endothelium because vasodilatation in response to endothelium-independent agonists such as NO, nitroglycerin, nitroprusside, and adenosine is not impaired.

Some insight has been gained into mechanisms that account for impairment of responses of cerebral vessels to endothelium-dependent agonists in the presence of pathophysiological states. During chronic hypertension and diabetes, altered responses of cerebral arterioles to endothelium-dependent agonists appear to be due to production of an endothelium-derived contracting factor (EDCF) that counteracts the normal dilator effect of NO. This EDCF in cerebral arterioles appears to be a cyclooxygenase product of arachidonic acid that activates a prostaglandin H\(_2\)/thromboxane A\(_2\) receptor.

During hypercholesterolemia, impaired endothelium-dependent relaxation of the basilar artery may be due to decreased production of NO, because administration of L-arginine, the substrate for NO synthase, restored dilator responses to acetylcholine toward normal.

Endothelium-dependent relaxation of large cerebral arteries is impaired after subarachnoid hemorrhage in experimental animals and humans. During hypercholesterolemia, impaired endothelium-dependent relaxation after subarachnoid hemorrhage is due to reduced formation of cGMP in response to EDCF. Other studies suggest that activity of guanylate cyclase and production of cGMP are normal.

Hemoglobin, which may play an important role in producing vasospasm after subarachnoid hemorrhage, may constrict cerebral arteries by inhibition of the basal influence of NO. In intact cerebral arteries, hemoglobin decreases basal levels of cGMP to levels similar to that produced by L-NAME or removal of endothelium. Hemoglobin avidly binds NO and thus prevents its entry into smooth muscle. In addition, hemoglobin may destroy NO by generation of superoxide anion.

Ischemia followed by reperfusion produces impaired endothelium-dependent responses of cerebral arterioles, which can be restored to normal with scavengers of oxygen radicals. Thus, formation of oxygen radicals, which can destroy NO, impairs endothelial function in cerebral arterioles after cerebral ischemia.
Nitric Oxide From Neurons

There appear to be two major neuronal sources of NO. The first is NO-producing neurons within the brain and spinal cord. The second is NO-producing perivascular fibers that innervate cerebral blood vessels.

Parenchymal Neurons

Nitric oxide synthase is also present in a subpopulation of neurons (up to approximately 2% of all neurons) throughout many regions of the brain. For neurons, some evidence suggests that staining for reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase is a reliable marker for activity of NO synthase under certain conditions. Studies using staining for NADPH-diaphorase suggest that NO synthase is present in dendrites and axon terminals closely associated with microvessels in the brain parenchyma. However, it is important to recognize the potential limitation of studies that rely exclusively on staining for NADPH-diaphorase.

Nitric oxide may mediate dilator responses of cerebral vessels in response to neuronal activation in brain. For example, increases in cerebral blood flow in response to activation of the fastigial nucleus of the brain. NO synthase-containing nerve fibers are also present in the wall of large and small cerebral arteries. In addition, astrocytes and microglia can release NO extracellularly in response to some stimuli. See text for references. L-Arg indicates L-arginine.

Perivascular Neurons

Immunochemistry for the neuronal form of NO synthase and staining for activity of NADPH-diaphorase suggest that large cerebral arteries and pial arterioles are innervated by NO synthase-containing nerve fibers in several species, including humans (Fig. 4). The source of these NO synthase-containing fibers appears to be the sphenopalatine ganglion. Neurons within this ganglion, which are a major source of innervation to cerebral arteries, stain intensely for NO synthase and NADPH-diaphorase. A number of functional studies indicate that activation of these fibers within the vessel wall, either electrically or with nicotine, produces NO-dependent relaxation in vitro. It is not clear which stimuli normally produce activation of these nerve fibers in vivo. Electric stimulation of parasympathetic fibers that originate from the sphenopalatine ganglion produces NO-mediated increases in cerebral blood flow. Recent evidence suggests that NO synthase-containing nerve fibers do not mediate increases in cerebral blood flow during stimulation of the fastigial nucleus or hypercapnia.

Nitric Oxide From Glia

Constitutive Nitric Oxide Synthase

Glial cells (astrocytes, microglia, and oligodendrocytes) are the predominant cell type in brain. Thus, production of NO by glia can potentially have a major impact on the cerebral vasculature. Recent studies indicate that L-arginine is found primarily in astrocytes.
in vivo, suggesting that glia may function as a cellular store for the substrate for NO production in brain.\textsuperscript{174} Astrocytes produce NO in response to several stimuli.\textsuperscript{175} A constitutive form of NO synthase is activated by receptor-mediated agonists in astrocytes (Fig 4).\textsuperscript{170-179} Immunocytochemical evidence suggests that levels of NO synthase in astrocytes may be lower than in neurons,\textsuperscript{174} and preliminary studies indicate that astrocytes in culture express messenger RNA (though at low levels) for constitutive NO synthase.\textsuperscript{180} It is possible that, like neurons, a constitutive form of NO synthase is only present in a subpopulation of astrocytes.

The constitutive NO synthase in astrocytes responds to several stimuli, including calcium ionophore and bradykinin and the neurotransmitters glutamate (via non-NMDA receptors) and norepinephrine.\textsuperscript{176-179} Bioassay studies suggest that activated astrocytes produce NO in quantities sufficient to produce relaxation of cerebral arteries.\textsuperscript{178,181} It is presently not known whether other glial cells (microglia and oligodendrocytes) express a constitutive form of NO synthase.

**Inducible Nitric Oxide Synthase**

There is now strong evidence that induction of NO synthase can occur in cultured astrocytes, microglia, C6 glioma cells, and human astrocytoma cells in response to lipopolysaccharide, some individual cytokines, and especially combinations of certain cytokines.\textsuperscript{182,183,189-191} This conclusion is based on several lines of evidence, including measurement of NO or nitrite, measurement of cGMP, measurement of citrulline production, and immunocytochemistry.\textsuperscript{175} An inducible form of NO synthase from rat astrocytes was cloned recently.\textsuperscript{192} Human immunodeficiency virus–coating glycoprotein gp 120 causes release of cytokines from glial cells and induction of NO synthase in astrocytoma cells.\textsuperscript{193} It is not known whether induction of NO synthase occurs in oligodendrocytes.

Relatively little is known regarding the regulation of induction of NO synthase in glia in vivo. Induction of the enzyme in astrocytes in response to lipopolysaccharide can be inhibited by dexamethasone, the neurotransmitter norepinephrine, and some cytokines (interleukin-4 and interleukin-10).\textsuperscript{180,188-189} Preliminary findings suggest that induction of NO synthase may occur in glia in vivo. For example, kainic acid\textsuperscript{190} and transient global ischemia with reperfusion\textsuperscript{196} induce high levels of NADPH-diaphorase in astrocytes in situ, which may reflect induction of NO synthase. A recent study detected messenger RNA for inducible NO synthase in brain of animals with conditions including rabies and encephalitis.\textsuperscript{197} It seems likely that at least a portion of this inducible NO synthase is expressed in glial cells.

**Other Vasoactive Stimuli**

### Hypercapnia

In addition to stimuli such as endothelium-dependent agonists and neuronal activation, other vasoactive stimuli can produce cerebral vascular responses that are dependent on production of NO. Hypercapnia is a potent dilator of cerebral blood vessels, and several studies suggest that cerebral vasodilatation during hypercapnia is dependent on formation of NO.\textsuperscript{130} This conclusion is based on the finding that increases in cerebral blood flow during hypercapnia are attenuated by inhibitors of NO synthase.\textsuperscript{89,153,198-203} Although these initial findings were obtained using rats, preliminary evidence suggests that increases in cerebral blood flow during hypercapnia are also dependent on formation of NO in dogs,\textsuperscript{204} cats,\textsuperscript{205} and rabbits.\textsuperscript{206} This inhibitory effect on vasodilatation during hypercapnia is not due to reductions in cerebral metabolism.\textsuperscript{200} Hypercapnia dilates cerebral blood vessels through a mechanism that requires development of extracellular acidosis,\textsuperscript{207} and L-NNA also inhibits increases in local cerebral blood flow in response to extracellular acidosis.\textsuperscript{202}

The precise role of NO in the cerebral vascular response to hypercapnia is not known. One possibility is that hypercapnia increases activity of NO synthase, and NO is the mediator that causes relaxation of vascular muscle. Consistent with this possibility is the finding that acidosis increases activity of isolated brain NO synthase.\textsuperscript{14} Another possibility is that NO is not the direct mediator of relaxation but that normal basal levels of NO and/or cGMP are required for the response to hypercapnia to occur.

It seems unlikely that formation of NO is the only mechanism involved, because increases in cerebral blood flow during very high levels of hypercapnia are not altered by an inhibitor of NO synthase and thus appear to be mediated by NO-independent mechanisms.\textsuperscript{203} Although a relatively high dose of L-NAME was used in this study,\textsuperscript{201} it is not clear whether complete inhibition of NO synthase was achieved. It is also important to note that although L-NMMA, L-NNA, and L-NAME inhibit the activity of NO synthase, the possibility that these compounds attenuate increases in cerebral blood flow during hypercapnia through an action unrelated to inhibition of NO synthase cannot be excluded.

Although vasodilatation during moderate hypercapnia appears to be dependent on production of NO, it is not clear which cell type(s) is responsible for production of NO. For example, relaxation of cerebral vessels occurs in vitro in response to hypercapnia (oracidosis)\textsuperscript{208-210} and has been observed to be endothelium independent in large cerebral arteries.\textsuperscript{208,209} However, these in vitro studies were performed using very high PCO\textsubscript{2} levels (>100 mm Hg), which may produce relaxation through NO-independent mechanisms.\textsuperscript{201} Whether the endothelium influences the response of isolated cerebral vessels to more moderate hypercapnia is not known. Vascular muscle is probably not a source of NO because constitutive NO synthase activity is not normally present in smooth muscle. NO synthase–containing nerve fibers are a potential source of NO, but in vivo studies suggest that these nerves do not mediate increases in cerebral blood flow during hypercapnia.\textsuperscript{86}

In vivo, light-induced injury of cerebral arterioles abolished vasodilatation in response to acetylcholine without affecting vasodilatation during hypercapnia.\textsuperscript{211} Because responses of cerebral arterioles to acetylcholine are endothelium dependent,\textsuperscript{3} these findings also suggest that endothelium is not a major source of NO during hypercapnia. Obviously, additional studies will be required to better define the role and source of NO that appear to be important for increases in cerebral blood flow to occur during hypercapnia.
In contrast to increases in cerebral blood flow during hypoxia, decreases in cerebral blood flow during hypocapnia are not altered by inhibitors of NO synthase.\textsuperscript{35,202}

**Hypoxia**

Initial studies suggested that increases in cerebral blood flow during hypoxia are not dependent on production of NO.\textsuperscript{80,198} A recent preliminary study suggests that formation of NO may not contribute to dilatation of cerebral arterioles during moderate hypoxia but may contribute during severe hypoxia.\textsuperscript{212}

**Autoregulation**

During moderate decreases in arterial pressure, cerebral blood vessels dilate to maintain cerebral blood flow relatively constant. Autoregulation of cerebral blood flow during such decreases in arterial pressure has been reported to be unaffected\textsuperscript{206,213} or impaired by inhibition of NO synthase.\textsuperscript{214} Thus, the role of NO in autoregulation of cerebral blood flow is unclear.

**Influence of Nitric Oxide During Ischemia**

Increased local concentrations of NO in brain have been measured during ischemia using an NO electrode\textsuperscript{215} and by electron paramagnetic resonance spin-trapping.\textsuperscript{216,217} The concentration of NO during ischemia began to increase within minutes after the onset of ischemia.\textsuperscript{215} Because this increase in the concentration of NO occurs relatively rapidly, it presumably reflects increased activity of constitutive NO synthase. However, as ischemia continues, levels of NO fall slowly but then increase again during reperfusion.\textsuperscript{215} Rapid increases in tissue concentrations of nitrite and cGMP as well as increases in citrulline production in the ipsilateral hemisphere after occlusion of the middle cerebral artery also support the concept that acute increases in NO production occur during cerebral ischemia.\textsuperscript{218} The cellular source(s) of increased NO production during ischemia and reperfusion is not known. Interestingly, a recent study suggests that ischemia causes increased expression of constitutive NO synthase in cerebral endothelium.\textsuperscript{219} Relying largely on the use of inhibitors of NO synthase, a number of studies have attempted to elucidate the impact of nitric oxide production during cerebral ischemia.\textsuperscript{130,220}

Nitric oxide may potentially have both beneficial and detrimental effects during cerebral ischemia.\textsuperscript{221} Beneficial effects of NO include maintenance of cerebral blood flow and inhibition of aggregation and adherence of platelets or leukocytes.\textsuperscript{219} Increased production of NO during ischemia may also be protective because NO has been shown to block NMDA receptors\textsuperscript{222} whose excessive activation may mediate cellular damage. Detrimental effects of excessive production of NO are possible because high concentrations of NO are cytotoxic (either directly or after combination with superoxide to form peroxynitrite).\textsuperscript{223} Studies using cultured neurons suggest that both NMDA- and glutamate-induced neurotoxicity\textsuperscript{199,224} and neuronal damage due to hypoxia\textsuperscript{225} may be mediated by NO.

In vivo studies evaluating the influence of NO on ischemia have obtained both positive and negative results.\textsuperscript{220} Several experiments with repeated administration of low doses of NO synthase inhibitors demonstrated a reduction of infarct volume and cerebral edema.\textsuperscript{227-229} Acute administration of NO synthase inhibitors has also been reported to be protective during focal cerebral ischemia in rats\textsuperscript{230,231} and selectively protective in the caudate nucleus in cats.\textsuperscript{84} Protection from ischemia was also observed after a single high dose of L-NAME given 15 hours before the onset of ischemia (produced by carotid occlusion in combination with hypoxia) in newborn rats.\textsuperscript{232}

In contrast to these studies, several others that used acute administration of NO synthase inhibitors have generally observed enhanced brain damage after ischemia.\textsuperscript{233-236} In addition, some in vivo studies report no effect by inhibitors of NO synthase on brain damage after ischemia.\textsuperscript{84,237,238} Although a completely clear pattern is not evident from the available studies, it has been suggested that production of NO may be beneficial in the first minutes or hours after the onset of ischemia but neurotoxic after many hours or days.\textsuperscript{220}

A number of factors may influence the extent of brain damage after ischemia, including changes in cerebral blood flow. For example, administration of L-arginine, the substrate for NO production, before and after occlusion of the middle cerebral artery in hypertensive rats dilation pial arterioles, increased cerebral blood flow, and reduced infarct size.\textsuperscript{239,240} These findings suggest that L-arginine may become a limiting factor for production of NO in brain during ischemia. In addition, infusion of sodium nitroprusside or 3-morpholino sydnonimine (SIN-1) (both NO donors) after occlusion of the middle cerebral artery increased local cerebral blood flow and reduced the volume of infarcted tissue.\textsuperscript{130,225} In contrast to these studies, however, administration of L-arginine did not reduce infarct volume in a similar model in a recent study.\textsuperscript{130} It is not known whether cerebral blood flow increased in response to L-arginine in the latter study.

Systemic administration of inhibitors of NO synthase increases arterial pressure in a dose-dependent manner\textsuperscript{2} and thus may increase blood flow in collateral-dependent regions of the brain after arterial occlusion. However, comparison of effects on arterial pressure is not possible because some studies that examined effects of inhibitors of NO synthase during ischemia did not measure arterial pressure.

Recently it has been proposed that the oxidation-reduction state of NO may determine whether NO is protective or destructive.\textsuperscript{241} Reduction of NO by superoxide is proposed to generate peroxynitrite ion, which is cytotoxic. In contrast, oxidation of NO produces nitrosonium ion, which can nitrosylate receptors for NMDA and thus prevent excessive stimulation of the receptor complex.\textsuperscript{222,241} One implication of this finding is that the redox state of the tissue may have a major influence on whether NO exerts beneficial or toxic effects.

It is possible that some damage to neurons during or after ischemia is mediated via activity of inducible NO synthase. Production of messenger RNA for cytokines such as interleukin-1β occurs in ischemic tissue\textsuperscript{242} and may provide a stimulus for induction of NO synthase. Unregulated overproduction of NO after ischemia may be directly cytotoxic. In addition, if L-arginine becomes a limiting factor in production of NO after ischemia, as has been suggested,\textsuperscript{239,240} then increased activity of NO
synthase in the absence of L-arginine may lead to production of superoxide anion and hydrogen peroxide, both of which are cytotoxic.\(^{11,14,16}\)

**Summary and Future Directions**

It is now clear that NO (or EDRF) plays a major role in regulation of the cerebral circulation. In addition to influencing basal tone and mediating endothelium-dependent relaxation, NO appears to mediate cerebral vasodilation in response to local neuronal activation. Thus, NO may be an important "coupler" of cerebral blood flow and metabolism.

Although recent studies have provided some insight into the potentially complex role of NO in the cerebral circulation (Fig 4), it seems likely that many additional effects of NO have yet to be defined. Relatively little is known regarding factors that regulate NO gene expression and activity of NO synthase in vivo. Regulation of expression of inducible NO synthase may be particularly important for pathophysiology because it is likely that this form of the enzyme is responsible for much of the cytotoxic effects of NO. The majority of studies addressing the influence of NO in cerebral vessels have relied exclusively on the use of inhibitors of NO synthase. Although this experimental approach has been very valuable, increasing incorporation of measurement of activity of NO synthase and NO itself in such experiments will be important.

Although production of NO appears to be important for cerebral vasodilation to occur during hypercapnia, the precise role and source of NO are not clear. The possible influence of NO synthase—containing perivascular nerves under physiological or pathophysiological conditions is also not known. The role of NO during cerebral ischemia is poorly understood at present because both beneficial and detrimental effects of increased NO production are possible. Clearly more studies are needed to further examine mechanisms by which NO production participates in regulation of the cerebral circulation.

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