Activation of Cerebellar Climbing Fibers Increases Cerebellar Blood Flow
Role of Glutamate Receptors, Nitric Oxide, and cGMP

Guang Yang, MD; Costantino Iadecola, MD

Background—The mechanisms regulating the cerebellar microcirculation during neural activity are poorly understood. One of the major neural inputs to the cerebellar cortex is the climbing fiber (CF), a pathway that uses excitatory amino acids, including glutamate, as a transmitter. We studied whether CF activation increases cerebellar blood flow (BFcrb) and, if so, we investigated the role of glutamate receptors, nitric oxide (NO) and cGMP, in the response.

Methods—The CF were activated by harmaline administration (40 mg/kg, IP) in halothane-anesthetized rats with a cranial window placed over the cerebellar vermis. BFcrb was monitored by a laser-Doppler probe, and arterial pressure and blood gases were controlled.

Results—With Ringer superfusion, harmaline produced sustained increases in BFcrb that peaked 20 minutes after administration (+115±13%; n=6; P<.05). The increases in BFcrb were substantially reduced by superfusion with tetrodotoxin (10 μmol/L; −91±5%; n=5; P<.05 from Ringer). The response was also attenuated by the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor inhibitor 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(F)-quinoxaline (100 μmol/L; −70±6%; P<.05; n=5), but not by the N-methyl-D-aspartate receptor blocker 2-amino-5-phosphonopentanoic acid (500 μmol/L; P>.05; n=5). The response was attenuated by the nonselective NO synthase (NOS) inhibitor nitro-L-arginine (1 mmol/L; −71±5%; n=6) or by 7-NI (50 mg, IP; −71±5%; n=5), a relatively selective neuronal NOS inhibitor. The soluble guanylyl cyclase inhibitor 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (100 μmol/L) attenuated the response to harmaline (−73±5; P<.05; n=6) but not to superfusion with adenosine (P>.05; n=5) or 8-bromo-cGMP (P>.05; n=5).

Conclusions—Activation of the CF system increases BFcrb. The response depends on activation of glutamate receptors and is in large part mediated by NO via stimulation of soluble guanylyl cyclase. Glutamate receptors NO and cGMP are important factors in the mechanisms of functional hyperemia in cerebellar cortex. (Stroke. 1998;29:499-508.)

Key Words: cerebellum ■ cerebral blood flow ■ glutamate antagonists ■ hypercapnia

The cerebellum, due to its relatively simple and well-characterized circuitry, is well suited to the investigation of the relationship between neural activity and blood flow. Functional brain imaging studies have demonstrated that BFcrb increases during motor and cognitive tasks, indicating that BFcrb is highly regulated and closely related to cerebellar neural activity.1–3 However, the mechanisms by which neural activity regulates BFcrb have not been studied as extensively as those of other brain regions (see references 4 and 5 for a review).

Two major excitatory synaptic inputs converge on cerebellar Purkinje cells, the only output neurons of the cerebellum: the PF and the CF. The PF are axons of cerebellar granule cells that reach to the superficial molecular layer and make synaptic contacts with Purkinje cell dendrites and molecular layer interneurons (see reference 6 for a review). The transmitter released from the PF is glutamate (see reference 7 for a review). The CF originate from the contralateral inferior olive and innervate Purkinje cell dendrites and interneurons.5 The transmitter released from the CF is also an excitatory amino acid, such as glutamate, aspartate, or N-acetyl-aspartyl-glutamate6,9

See Editorial Comment, page 507 (see reference 10 for a review). The interaction between CF and PF activity modulates Purkinje cells output and is responsible for long-term depression, a phenomenon thought to subserve cerebellar plasticity and learning.11 The mechanisms by which activation of the different inputs to the cerebellar cortex influences local blood flow have not been fully elucidated. Although studies in which the PF were electrically stimulated have provided an insight into the role of this system in the regulation of BFcrb,12–17 PF activity is unlikely to be the sole determinant of BFcrb during normal cerebellar function. The CF have a powerful synaptic associ-
Materials and Methods

General Surgical Procedures

Experimental protocols were approved by the Institutional Animal Care Committee. Studies were performed on 81 male Sprague-Dawley rats (Sasco, Omaha, Neb) weighing 290 to 380 g. Rats were anesthetized with 5% halothane in 100% oxygen. After induction of anesthesia, the concentration of halothane was reduced to 1%. Because animals were not paralyzed, the level of anesthesia was assessed by testing corneal reflexes and motor responses. Anesthesia was maintained at 60% using a respirator for 2 to 3 minutes and the changes in BFcrb were monitored. In other rats (n=5), the effect of the inactive isomer of nitro-arginine D-NA on the BFcrb response to harmaline was tested. Multiple injections of glutamate using this technique have been shown to elicit reproducible increases in BFcrb.

Effect of Nitro-Arginine on the Increases in BFcrb Produced by Harmaline

Harmaline (40 mg/kg, IP) was administered, and the changes in BFcrb were monitored continuously for up to 90 minutes after administration. In one group of rats (n=6), the window was superfused with Ringer. Arterial blood gas values were then adjusted. Studies commenced when arterial pressure, arterial blood gas values, and flow signal were in a steady state. In these experiments the BFcrb were activated by systemic hypercapnia (PCO2 50 to 60 mm Hg) was tested while the window was superfused with Ringer. CO2 was introduced into the circuit of the respirator for 2 to 3 minutes and the changes in BFcrb were monitored.11 After PCO2 had returned to baseline, TTX (10 μmol/L; n=5), NQBX (100 μmol/L; n=5), or AP-5 (500 μmol/L; n=5) was superfused for 30 minutes, and the reactivity of BFcrb to hypercapnia was tested again. Drugs were dissolved in Ringer and were applied at concentrations found to be effective in previous studies.12,13 In another group of rats (n=6), the effect of AP-5 on the increase in BFcrb produced by glutamate was tested. Glutamate (200 nmol/200 nL) was microinjected into the cerebellar cortex using a micropipette connected to a pressurized microinjection system, and the resulting changes in BFcrb were monitored (see reference 16 for a detailed description). AP-5 (500 μmol/L) was then superfused for 30 minutes, and the effect of glutamate microinjection was tested again. Multiple injections of glutamate using this technique have been shown to elicit reproducible increases in BFcrb.

Effect of NOS Inhibitors on the Increases in BFcrb Produced by Harmaline

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## Arterial Pressure and Blood Gas Values of the Rats Studied

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<th>AP (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>pH</th>
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<td>125±6</td>
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</table>

AP indicates arterial pressure.

*Parameters refer to the time point when the BFcrb increases were greatest; P > .05 between before and after pairs (paired t test).
Bfcrb produced by topical application of the NO donor SNAP (100 μmol/L) or of adenosine (1 mmol/L) was tested. Drugs were topically applied until the increase in Bfcrb reached a steady state (usually 3 to 5 minutes).

Effect of 7-NI on the Increases in Bfcrb Produced by Harmaline
The relatively selective neuronal NOS (nNOS) inhibitor 7-NI (50 mg/kg, IP; in oil) was administered 30 minutes before harmaline. This concentration of 7-NI reduced cerebellar NOS activity by ~70%.

The effect of vehicle (oil; n=5) on the elevations in Bfcrb produced by harmaline and the effect of 7-NI on the increase in Bfcrb produced by hypercapnia (n=6) or topical application of SNAP (100 μmol/L; n=5) were studied in separate groups of rats.

Effect of ODQ on the Increase in Bfcrb Produced by Harmaline
The soluble guanylyl cyclase inhibitor ODQ (100 μmol/L) was superfused on the cranial window for 45 minutes and then harmaline was administered (n=5). ODQ was dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the solution was adjusted to be less than 0.05%. In five rats the effect of ODQ on the vasodilation produced by SNAP (100 μmol/L), adenosine (1 mmol/L), or the cGMP analogue 8-Br-cGMP (100 μmol/L) was studied. In five additional rats the effect of ODQ on the vasodilation produced by hypercapnia was tested.

Data Analysis
Data in text, the table, and figures are presented as mean±SE. Comparisons between two groups were evaluated by paired or unpaired Student’s t tests as appropriate. Multiple comparisons were evaluated by ANOVA and Tukey’s test (Systat Inc.).

Results
Effect of Harmaline on Bfcrb
Administration of harmaline (40 mg/kg, IP) produced fine tremors restricted to the whiskers and the facial muscles. Harmaline elicited a profound increase in Bfcrb that began 5 minutes after administration, peaked between 15 and 20 minutes later (+115±13%; n=6), and was still present (+25±7%) at 90 minutes (Fig 1A; P<.05 from time zero; ANOVA and Tukey’s test). The increases in Bfcrb were not associated with changes in arterial pressure or blood gas values (Fig 1B; Table).

Effect of TTX and Glutamate Receptor Inhibition on the Increase in Bfcrb Produced by Harmaline

To determine whether the increases in flow were related to synaptic activity, the effect of TTX was studied. TTX (10 μmol/L; n=5) produced a small but significant reduction in resting Bfcrb (before: 11.0±0.7, after: 9.0±0.3 perfusion units; P<.05; t test; n=5) and attenuated substantially the increase in Bfcrb elicited by harmaline (P<.05 from Ringer at each time point) (Fig 1A). Twenty minutes after administration of harmaline, the Bfcrb increase was attenuated by 95±5% (P<.05) (Fig 1A). However, TTX did not affect the increase in flow produced by hypercapnia (Ringer’s: +69±8%; TTX: +71±8%; P>.05; n=5).

Excitatory amino acids mediate synaptic transmission in the CF system. Therefore, we investigated whether the increase in Bfcrb produced by harmaline was related to activation of glutamate receptors. Superfusion with the AMPA receptor antagonist NBQX (100 μmol/L) did not affect resting Bfcrb (before: 8.2±0.3; after: 7.6±0.3 perfusion units; P>.05; t test; n=5), but attenuated substantially the Bfcrb response to harmaline (P<.05; n=5) (Fig 2A). NBQX did not affect the increase in Bfcrb produced by hypercapnia (before: +58±5%; after: +68±5%; P>.05; n=5). In contrast, the NMDA receptor antagonist AP-5 (500 μmol/L) did not attenuate the increase in Bfcrb produced by harmaline or hypercapnia (Fig 2; P>.05 from Ringer, ANOVA; n=5). However, AP-5 inhibited the increase in Bfcrb-produced

<table>
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<th>Treatment</th>
<th>AP (mm Hg)</th>
<th>Pco2 (mm Hg)</th>
<th>PO2 (mm Hg)</th>
<th>pH</th>
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</table>
microinjection of glutamate (200 nmol/200 nL) into the cerebellar molecular layer (Fig 2B; \(P<.05; n=6\)).

**Effect of NOS Inhibition on the Increases in BFcrb Produced by Harmaline**

The data presented above indicate that the increase in BFcrb produced by harmaline is mediated by activation of a glutamate receptor. Because glutamate receptor activation increases blood flow through NO production,\(^{14,24–26}\) we studied the effect of NOS inhibitors on the vascular response to harmaline administration. Superfusion with the nonselective NOS inhibitor L-NA (1 mmol/L) reduced resting BFcrb (before: 10.5±0.4; after: 8.9±0.4 perfusion units; \(P<.05; t\) test; \(n=5\)) and attenuated the increase in BFcrb produced by harmaline by 73±5% (\(P<.05; n=5\)) (Fig 3A). L-NA also attenuated the BFcrb response to hypercapnia (\(-48±10\%; P<.05\)), but not to topical application of the NO donor SNAP or adenosine (\(P>.05; n=5\)) (Fig 3B). In contrast, the inactive isomer D-NA did not affect the increase in BFcrb produced by harmaline (Fig 3A; \(P>.05\) from Ringer; ANOVA; \(n=5\)). Others have reported that L-NA attenuates the increase in BFcrb produced by adenosine.\(^{27}\) However, in our experimental preparation adenosine behaved as a NO-independent vasodilator. The observation that ODQ does not affect the response to adenosine also supports this conclusion (see below).

To study the role of nNOS in the response, the relatively selective nNOS inhibitor 7-NI was tested. We have previously demonstrated that in this preparation 7-NI inhibits nNOS without affecting eNOS-dependent vascular responses.\(^{14,16}\) 7-NI reduced resting BFcrb (before: 8.7±0.6; after: 6.3±0.3 perfusion units; \(P<.05; t\) test; \(n=6\)) and attenuated the increase in BFcrb produced by harmaline (\(-71±5\%; P<.05; n=6\)) or hypercapnia (\(-63±6\%; P<.05; n=6\)) but not to SNAP superfusion (\(P>.05; n=5\); Fig 4). Thus, the BFcrb response to harmaline is attenuated by both L-NA and 7-NI.

**Effect of the Guanylyl Cyclase Inhibitor ODQ on the Increase in BFcrb Produced by Harmaline**

One of the mechanisms by which NO produces vascular relaxation is by activating soluble guanylyl cyclase and increasing cGMP in vascular smooth muscles (see reference 28 for a review). Therefore, in these experiments we studied the effect of the soluble guanylyl cyclase inhibitor ODQ on the increase in BFcrb produced by harmaline. ODQ (100 μmol/L) increased resting BFcrb (before: 10.0±0.7; after: 12.0±0.9 perfusion units; \(P<.05; t\) test; \(n=6\)) and inhibited the response to harmaline (\(-73±5\%; P<.05; n=6\)) (Fig 3A) or hypercapnia (\(-49±7\%; P<.05; n=6\)) (Fig 5). ODQ attenuated the increase in BFcrb produced by SNAP (\(P<.05; n=6\)), but did not affect the response to the cAMP-dependent vasodilator adenosine (\(P>.05; n=5\)) or to the cGMP analogue 8-Br-cGMP (\(P>.05; n=6\)) (Fig 5). The finding that ODQ did not affect the vasodilation produced by adenosine supports the hypothesis that the vasodilation produced by this nucleoside is independent of NO and cGMP.

**Discussion**

We have investigated the mechanisms of the increases in BFcrb produced by activation of the CF. The CF provide a strong excitatory synaptic input to the cerebellar Purkinje cells. The CF originate from the contralateral inferior olive, project directly to the cerebellar molecular layer, and make multiple synaptic contacts with Purkinje cell dendrites and molecular layer interneurons.\(^{5}\) Despite the fact that a Purkinje cell receives inputs only from a single CF,\(^{6}\) CF activation produces powerful synaptic responses in Purkinje cell dendrites \(^{29}\) (see reference 30 for a review). CF-induced Purkinje cell discharges are associated with increases in cerebellar glucose utilization.\(^{31}\) We have found that activation of the CF using harmaline elicits profound increases in BFcrb that are independent of changes in arterial pressure and blood gases. The increases in BFcrb are protracted in time and are larger in magnitude than those produced by stimulation of the PF, hypercapnia, or topical application of vasodilators.\(^{13–16,32}\) The flow increase is virtually abolished by TTX, indicating that it is mediated by enhanced synaptic activity. The latter finding also suggests that direct vascular effects of harmaline are unlikely to contribute to the
vasodilation. These observations indicate that the synaptic activity evoked from the CF has profound effects on the microvascular flow of the cerebellar cortex and that CF activity is a major determinant of BFcrb. Harmaline could also activate other neural pathways in addition to the CF. However, mapping of neural activity either by microelectrode recordings or 2-deoxyglucose autoradiography suggests that the activation produced by harmaline involves predominantly the inferior olive–CF system. It is, therefore, likely that the increases in BFcrb produced by harmaline reflect largely CF activity. The data provide evidence that CF activity is an important factor in the elevations in BFcrb that occur during normal cerebellar function.

We then began to study the mechanisms of the increase in flow evoked by CF activation. Synaptic transmission in the CF system is mediated by excitatory amino acids, which act on glutamate receptors on Purkinje cell dendrites and interneurons. We, therefore, tested the hypothesis that activation of glutamate receptors initiates the increase in flow produced by CF activation. It was found that the vascular response to harmaline is attenuated by NBQX, an AMPA receptor blocker, but not by the NMDA receptor blocker AP-5. The lack of effectiveness of AP-5 could not be attributed to insufficient dose or poor penetration of the drug because AP-5 attenuates the increase in BFcrb produced by glutamate microinjection. These observations suggest that the increases in flow evoked from CF activation are mediated largely by activation of AMPA receptors. The observation that NBQX does not block the flow increase completely raises the possibility that metabotropic glutamate receptors or other receptors are also involved in the response. Metabotropic glutamate receptors are present on Purkinje cell dendrites, and they are linked to NO production. However, future studies are required to define the role of these receptors in the flow response to harmaline.

Figure 2. A, Effect of glutamate receptor antagonists on the increase in BFcrb produced by harmaline. The NMDA receptor inhibitor AP-5 does not affect the increase in BFcrb (P > .05 from Ringer superfusion; ANOVA) (see Fig 1 for BFcrb response during Ringer superfusion). In contrast, the AMPA receptor inhibitor NBQX attenuates the increase in flow substantially (P < .05 from AP-5 at each time point; t test). B, The NMDA receptor antagonist AP-5 does not affect the vasodilation produced by hypercapnia (Pco2 = 50 to 60 mm Hg; see Table for values) but attenuates the increase in BFcrb produced by microinjection of glutamate into the cerebellar molecular layer. The data provide evidence that AP-5 is effective in inhibiting glutamate receptors.

Figure 3. A, Effect D-NA, L-NA and ODQ on the increases in BFcrb produced by hypercapnia. The inactive stereoisomer of nitroarginine, D-NA, does not affect the response (P > .05 from Ringer; ANOVA) (see Fig 1 for BFcrb response during Ringer superfusion). The nonselective NOS inhibitor L-NA and the guanylyl cyclase inhibitor ODQ attenuate the response substantially (P < .05 from D-NA; ANOVA and Tukey’s test). The data suggest that the increase in BFcrb produced by harmaline is, in great part, mediated by NO and cGMP. B, L-NA attenuates the increase in BFcrb produced by hypercapnia (P < .05; t test) but does not reduce the response to topical application of the NO donor SNAP or of the NO-independent vasodilator adenosine.
Therefore, we studied whether NO, a potent vasodilator, participates in the increase in BFcrb produced by harmaline. The BFcrb response to harmaline was attenuated by the NOS inhibitor L-NA and by the relatively selective inhibitor of nNOS 7-NI. The effect of L-NA or 7-NI could not be attributed to a nonspecific loss of vascular reactivity because these agents did not affect the increase in BFcrb produced by the NO donor SNAP or by the NO-independent vasodilator adenosine. L-NA has been reported to also inhibit ATP-sensitive K⁺ channels. The possibility that activation of K⁺ channels contributes to the vasodilation cannot be ruled out on the basis of the present study. However, 7-NI, a NOS inhibitor that is structurally unrelated to L-NA and that inhibits NOS by a mechanism different from that of L-NA, attenuates the BFcrb response to harmaline in a fashion nearly identical to that of L-NA. This observation supports the notion that the effect of L-NA is stereoselective and that it provides additional support to the contention that the attenuation of the response by L-NA is related to NOS inhibition.

One of the mechanisms by which NO produces vasodilation is activation of soluble guanylyl cyclase and cGMP production (see reference 28 for a review). To determine whether cGMP is involved in the vasodilation produced by harmaline, we used the recently introduced soluble guanylyl cyclase inhibitor ODQ. It was found that ODQ attenuates the BFcrb response to harmaline without affecting the vasodilation produced by the cGMP analogue 8-Br-cGMP or the guanylyl cyclase-independent vasodilator adenosine. These observations indicate that the effect of ODQ is related to guanylyl cyclase inhibition. The finding that the attenuation by ODQ of the BFcrb to harmaline is virtually identical in magnitude to that produced by NOS inhibition suggests that the NO-dependent component of the response is mediated by activation of guanylyl cyclase and not by other effects of NO, resulting in smooth muscle relaxation. The cells responsible for the production of NO during CF activation remain to be defined. The observation that L-NA and 7-NI attenuate the response to harmaline administration by a similar degree is consistent with a neuronal source of NO. In the cerebellar molecular layer, NOS is present in interneurons, mainly basket and stellate cells, but not in Purkinje cells. It is, therefore, likely that NO is produced by molecular layer interneurons.

The evidence presented above suggests the following mechanism for the increase in BFcrb produced by CF activation. CF activation produces depolarization of Purkinje cells and interneurons. The associated increase in intracellular calcium activates NOS in interneurons, resulting in production of NO. NO, or a closely related chemical specie, diffuses to local blood vessels and activates soluble guanylyl cyclase, resulting in vasodilation. However, NO is unlikely to be the sole factor responsible for the vasodilation. The observations that NOS or guanylyl cyclase inhibition attenuates but does not abolish the increase in BFcrb produced by harmaline suggests that a component of the vasodilation is independent of NO/cGMP. However, the component of the response independent of NO is relatively small. The mechanisms of such NO-independent...
component remain to be defined. In the PF system, adenosine is responsible for the portion of the vasodilation not mediated by NO.\textsuperscript{17} Adenosine, a potent cerebrovasodilator,\textsuperscript{49} is present in Purkinje cells\textsuperscript{20} and could conceivably be released also during CF-induced synaptic activity. However, the role of adenosine in the response will have to be addressed in future studies.

Stimulation of the PF increases BF\textsubscript{crb}, an effect that is also thought to be mediated by glutamate receptors and, in part, NO.\textsuperscript{12,13,17} However, there are important differences between the BF\textsubscript{crb} response evoked from PF or CF stimulation. First, the magnitude of the flow increase produced by CF activation (\(\approx 100\%\)) is greater than that produced by PF stimulation (\(\approx 50\%\)). The larger flow response is likely to reflect the strong synaptic interaction between CF and Purkinje cells and interneurons.\textsuperscript{29} Second, the part of the NO-dependent component of the vasodilation elicited by CF activation (\(\approx 70\%\)) is larger than that of the vasodilation produced by PF stimulation (\(\approx 50\%\)). This observation suggests that the contribution to the flow response of molecular layer interneurons, the presumed cellular source of NO, is greater during activation of the CF than the PF. However, the possibility that these differences are related to the method used to activate these pathways, electrical stimulation for the PF versus harmaline for the CF, cannot be ruled out.

Another new finding of the present study is that the guanylyl cyclase inhibitor ODQ attenuates the response to hypercapnia. NO has been implicated in the mechanisms of the vasodilation produced by hypercapnia in several species.\textsuperscript{51-55} However, the lack of selective and specific guanylyl cyclase inhibitors precluded the need to test more directly the role of cGMP in the response. Commonly used guanylyl cyclase inhibitors, such as methylene blue and LY83583, are not suitable because they also inactivate NO by producing reactive oxygen species.\textsuperscript{56} Furthermore, methylene blue inhibits NOS directly.\textsuperscript{57} ODQ is a guanylyl cyclase inhibitor that does not have the drawbacks of methylene blue or LY83583.\textsuperscript{22,23} The observation that ODQ attenuates the hypercapnic vasodilation provides evidence that cGMP production is required for a sizable component of the flow response and provides additional support to the hypothesis that NO is involved in the mechanisms of the hypercapnic vasodilation.

In summary, we have demonstrated that activation of the cerebellar CF by harmaline increases local blood flow substantially. The effect is markedly reduced by TTX and by NBQX, suggesting that the response is mediated by glutamatergic synaptic transmission. In addition, the flow increase is attenuated by the NOS inhibitors L-NA and 7-NI or by the guanylyl cyclase inhibitor ODQ. Collectively, the data indicate that the increase in BF\textsubscript{crb} produced by harmaline is initiated by excitatory amino acids released from the CF through the production of NO and cGMP. We conclude that CF activity is an important factor in the local control of blood flow in the cerebellar cortex and that glutamate, NO, and cGMP are critical mediators in the regulation of flow during neural activity in the cerebellar cortex.

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References

**Editorial Comment**

Although it is well known that increases in neuronal activity are associated with local increases in perfusion, the mechanism(s) that mediates this increase in cerebral blood flow has been difficult to fully define. A series of studies over the last 9 years support the concept that neuronal release of the potent vasodilator nitric oxide (NO) functions to increase local blood flow.

Relative to other organs, the brain produces large quantities of NO. Under normal conditions, the majority of this NO is produced by the neuronal isoform of NO-synthase (nNOS). A major stimulus for production of NO by nNOS in neurons is activation of glutamate receptors. For example, recent molecular analysis revealed physical coupling (via post-synaptic density proteins) of nNOS to one subtype of glutamate receptor, the NMDA receptor. Activation of NMDA receptors increases activity of nNOS, while inhibitors of NMDA receptors or NO-synthase attenuate basal and stimulated NO production in a variety of models, including brain of awake animals (see references 4 and 5 for examples).

Because NO is lipid- and water-soluble, it can easily diffuse extracellularly and influence local vascular tone. Using a bioassay system, Garthwaite et al provided the first direct evidence that neurons release NO in response to activation of NMDA receptors in quantities sufficient to relax vascular muscle in vitro. We subsequently reported that local dilatation of cerebral arterioles in response to activation of NMDA receptors in vivo is mediated by NO. Since our initial observation, several other laboratories have confirmed that glutamate or glutamate analogues produce NO-mediated vasodilation in brain (see references 8–12 for examples).

The study presented here makes an important contribution in this area. Previous in vivo studies that focused on cerebral vascular responses have been performed almost exclusively in the cerebral cortex. In this study, the experimental approach took advantage of the fact that the neuronal circuitry and...
neurochemistry of the cerebellar cortex are simpler than that in many other brain regions and have been relatively well characterized. The results indicate that increases in cerebellar blood flow in response to activation of climbing fibers (using harmaline) was inhibited by an antagonist of the AMPA subtype of glutamate receptor and inhibitors of NO-synthase (including one selective for nNOS). The approach using harmaline is attractive because it allows one to examine mechanisms that mediate vascular responses to endogenous release of an excitatory amino acid which activates glutamate receptors. Thus endogenous neurotransmitter release and subsequent activation of glutamate receptors produced NO-mediated vasodilatation.

An additional goal was to examine the mechanism by which NO increased blood flow. Soluble guanylyl cyclase in vascular muscle is known to be a key molecular target for NO. Previous studies have provided evidence that relaxation of cerebral vessels to NO, produced endogenously by endothelium or perivascular neurons, is mediated in large part by activation of soluble guanylyl cyclase.\textsuperscript{13,14} In the present study, increases in cerebellar blood flow in response to activation of climbing fibers was also reduced markedly by a selective inhibitor of soluble guanylyl cyclase. Thus these findings provide additional support for the concept that vasodilatation in brain in response to activation of glutamate receptors is mediated by neuronally derived NO acting on soluble guanylyl cyclase in cerebral vascular muscle.

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