Nitric oxide is the predominant mediator of cerebellar hyperemia during somatosensory activation in rats

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Yang, Guang, Gang Chen, Timothy J. Ebner, and Costantino Iadecola. Nitric oxide is the predominant mediator of cerebellar hyperemia during somatosensory activation in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1760–R1770, 1999.—Crus II is an area of the cerebellar cortex that receives trigeminal afferents from the perioral region. We investigated the mechanisms of functional hyperemia in cerebellum using activation of crus II by somatosensory stimuli as a model. In particular, we sought to determine whether stimulation of the perioral region increases cerebellar blood flow (BFcrb) in crus II and, if so, whether the response depends on activation of 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-kainate receptors and nitric oxide (NO) production. Crus II was exposed in anesthetized rats, and the site was superfused with Ringer. Field potentials were recorded, and BFcrb was measured by laser-Doppler flowmetry. Crus II was activated by electrical stimulation of the perioral region (upper lip). Perioral stimulation evoked the characteristic field potentials in crus II and increased BFcrb (34 ± 6%; 10 Hz-25 V; n = 6) without changing arterial pressure. The BFcrb increases were associated with a local increase in glucose utilization (74 ± 8%; P < 0.05; n = 5) and were attenuated by the AMPA-kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (-71 ± 3%; 100 µM; P < 0.01; n = 5). The neuronal NO synthase inhibitor 7-nitroindazole (7-NI, 50 mg/kg; n = 5) virtually abolished the increases in BFcrb (-90 ± 2%; P < 0.01) but did not affect the amplitude of the field potentials. In contrast, 7-NI attenuated the increase in neocortical cerebral blood flow produced by perioral stimulation by 52 ± 6% (P < 0.05; n = 5). We conclude that crus II activation by somatosensory stimuli produces localized increases in local neural activity and BFcrb that are mediated by activation of glutamate receptors and NO. Unlike in neocortex, in cerebellum the vasodilation depends almost exclusively on NO. The findings underscore the unique role of NO in the mechanisms of synaptic function and blood flow regulation in cerebellum.

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METHODS

Methods for monitoring blood flow using laser-Doppler flowmetry (LDF) for recording of field potentials and for determination of GU have been described previously (16, 17) and are summarized below.

General Surgical Procedures

Studies were performed on 58 male Sprague-Dawley rats (Sasco, Omaha, NE) weighing ~300 g. Rats were anesthetized with 5% halothane in an oxygen-nitrogen mixture. After induction of anesthesia, the concentration of halothane was reduced to 1–2%. Catheters were inserted in both femoral arteries, in the left femoral vein, and in the trachea. Animals were then placed on a stereotaxic frame (model 1404; D. Kopf Instruments, Tujunga, CA) mounted on a vibration-free table (TMC, Peabody, MA) and artificially ventilated with a oxygen-nitrogen mixture by a mechanical ventilator (model 638; Instruments, Tujunga, CA) maintained at 37°C. Arterial pressure and arterial blood gases (PaO2, arterial partial CO2 pressure; PaCO2, arterial partial O2 pressure; 7-NI, 7-nitroindazole; L-NA, nitro-L-arginine; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one. P > 0.05 ANOVA and Tukey’s test. *15 min after injection of 2-deoxyglucose.

Table 1. Arterial pressure and arterial blood gases in rats subjected to perioral stimulation

<table>
<thead>
<tr>
<th>MAP, mmHg</th>
<th>PaCO2, mmHg</th>
<th>PaO2, mmHg</th>
<th>pH</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Crus II</td>
<td>103 ± 3</td>
<td>34.8 ± 1.3</td>
<td>163 ± 9</td>
<td>7.42 ± 0.03</td>
</tr>
<tr>
<td>nBOX</td>
<td>101 ± 2</td>
<td>33.9 ± 1.3</td>
<td>152 ± 11</td>
<td>7.51 ± 0.02</td>
</tr>
<tr>
<td>After</td>
<td>100 ± 2</td>
<td>35.8 ± 0.9</td>
<td>138 ± 17</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>Oil</td>
<td>103 ± 3</td>
<td>34.1 ± 1.6</td>
<td>151 ± 14</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>After</td>
<td>104 ± 2</td>
<td>34.4 ± 0.8</td>
<td>144 ± 5</td>
<td>7.49 ± 0.01</td>
</tr>
<tr>
<td>7-NI</td>
<td>102 ± 1</td>
<td>36.1 ± 1.3</td>
<td>146 ± 6</td>
<td>7.48 ± 0.02</td>
</tr>
<tr>
<td>After</td>
<td>102 ± 3</td>
<td>35.4 ± 0.6</td>
<td>138 ± 8</td>
<td>7.50 ± 0.01</td>
</tr>
<tr>
<td>L-NA</td>
<td>103 ± 2</td>
<td>35.8 ± 1.0</td>
<td>151 ± 10</td>
<td>7.49 ± 0.01</td>
</tr>
<tr>
<td>After</td>
<td>105 ± 4</td>
<td>36.1 ± 1.2</td>
<td>151 ± 5</td>
<td>7.48 ± 0.01</td>
</tr>
<tr>
<td>ODQ</td>
<td>103 ± 2</td>
<td>35.8 ± 0.6</td>
<td>140 ± 12</td>
<td>7.48 ± 0.02</td>
</tr>
<tr>
<td>After</td>
<td>102 ± 1</td>
<td>34.2 ± 1.1</td>
<td>140 ± 8</td>
<td>7.48 ± 0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>100 ± 3*</td>
<td>34.4 ± 1.3*</td>
<td>172 ± 3</td>
<td>7.43 ± 0.03*</td>
</tr>
<tr>
<td>utilization</td>
<td>100 ± 3*</td>
<td>34.4 ± 1.3*</td>
<td>172 ± 3</td>
<td>7.43 ± 0.03*</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>107 ± 4</td>
<td>33.6 ± 0.8</td>
<td>131 ± 4</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>stimulation parameters</td>
<td>107 ± 4</td>
<td>33.6 ± 0.8</td>
<td>131 ± 4</td>
<td>7.49 ± 0.02</td>
</tr>
<tr>
<td>7-NI</td>
<td>109 ± 4</td>
<td>38 ± 0.5</td>
<td>161 ± 17</td>
<td>7.49 ± 0.01</td>
</tr>
<tr>
<td>After</td>
<td>113 ± 2</td>
<td>34.2 ± 1.3</td>
<td>155 ± 10</td>
<td>7.48 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. MAP, mean arterial pressure; PaCO2, arterial partial CO2 pressure; PaO2, arterial partial O2 pressure; 7-NI, 7-nitroindazole; L-NA, nitro-L-arginine; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one. P > 0.05 ANOVA and Tukey’s test. *15 min after injection of 2-deoxyglucose.

Perioral Stimulation and Monitoring of Blood Flow and Field Potentials

A small craniotomy (3 × 3 mm) was performed in the interparietal bone using a dental drill. The dura was carefully removed, and the left cerebellar hemisphere was exposed (crus I and II). In studies in which CBF was monitored in the somatosensory cortex, a small craniotomy (3 × 3 mm) was performed 2–3 mm posterior to bregma and 6 mm lateral to the midline. The cranial window was continuously superfused with Ringer or drugs at a rate of 0.33 ml/min (see Ref. 15 for Ringer composition and Ref. 17). The turnover rate of the window volume was 20–30 s. As in previous studies, solutions were equilibrated with 100% O2 and 5% CO2 (pH 7.3–7.4) and warmed to 37°C (17). Two needle electrodes (distance between electrodes: 0.5 cm) were inserted in the perioral region (upper lip) for delivering low-intensity electric stimuli (5–30 V; 2–16 Hz; see Ref. 4). For BFst studies, stimuli were negative square waves (pulse duration 0.3 ms) delivered from a Grass S88 stimulator through a stimulus isolation unit (P51U6; Grass).

Field potentials were recorded in crus II by glass microelectrodes (tip diameter 30–50 µm) filled with 2 M NaCl (resistance 2–5 MΩ) and were inserted at a depth of ~400 µm. Another micropipette served as reference electrode. The signal from the micropipettes was fed through an extracerebral microelectrode amplifier (7P5; Grass), displayed on an oscilloscope, and digitized using a computerized data acquisition system (MacAdios IIjr; GW Instruments). In studies of field potentials, PF were stimulated at the rate of 1/s (100 µA; pulse duration 0.3 ms). In each trial, 20 traces were acquired, averaged, and stored for off-line analysis (Superscope software; GW Instruments).

CBF in somatosensory cortex and BFst in crus II were monitored using a laser-Doppler flowmeter (model BPM 403A; Vasamedic Flowmeter, St. Paul, MN; see Ref. 17). The LDF probe (tip diameter 0.8 mm) was mounted on a micromanipulator (Kopf) and was positioned 0.5 mm above the pial surface. The analog output of the flowmeter was fed into a direct current amplifier (model 7P1; Grass) and displayed on the polygraph. To avoid pulsatile variations in the flow signal, a long time constant was used (5 s). After a 10- to 20-min stabilization period, probe position and reactivity of the preparation were tested at each site by determining the cerebrovascular reactivity to inhalation of 5% CO2. Changes in flow were calculated as percentage of the baseline value determined at the end of the experiment.

Cerebral GU

GU was determined by the 2-[14C]deoxyglucose (2-DG) method with quantitative autoradiography (32). As described in detail elsewhere (17), 2-DG (12.5 µCi/100 g in 1 ml 0.9% NaCl; New England Nuclear) was infused intravenously for 40 s using an infusion pump. Simultaneously, ~70 µl of arterial blood were collected every 5–10 s during the first minute, at 90 s, and at 2, 3, 5, 10, 15, 25, 35, and 45 min. Blood samples were centrifuged and stored on ice. Forty-five minutes later, rats were killed by an intravenous bolus of saturated KCl. The brain was then rapidly removed and frozen in isopentane cooled to −30°C with dry ice. Serial sections (20 µm) were cut through the cerebellum using a cryostat (Hacker-Bright). Sections were picked up from the blade with coverslips, mounted on glass slides, and exposed to X-ray film (DuPont) together with calibrated 14C standards (17). Ten days later, the film was developed using an automatic developer (Kodak), and the optical density (OD) of regions of interest was determined bilaterally on four adja-
cent sections using a computerized image analyzer (MCID system; Imaging Research). At the site of stimulation, OD was measured in all of the sections in which a focal increase in density was observed. OD was transformed in 14C concentration (nCi/g) using the standards on the film (17). Radioactivity (nCi/g) of plasma samples was determined by liquid scintillation counting as previously described (17). Plasma glucose was measured using a glucose analyzer (Beckman). GU (µmol·100 g⁻¹·min⁻¹) was calculated from the OD of the regions of interest and the arterial time course of 2-DG using the equation developed by Sokoloff et al. (32).

Experimental Protocol

Relationship between stimulus parameters and blood flow in cerebellar and cerebral cortex. In these experiments (n = 6), the cranial window was superfused with Ringer. Stimulating electrodes were inserted, and the LDF probe was placed on crus II. The preparation was then allowed to stabilize. Arterial blood gases were adjusted and maintained in a steady state (Table 1). The periroral region was stimulated for 40 s, and the corresponding BFcrb increases were recorded in ipsilateral crus II. Multiple stimulations were delivered, and at each stimulation the intensity of the stimulus was varied (5–30 V) while the stimulus frequency was maintained at 10 Hz. Next, the frequency of the stimulus was increased (2–16 Hz) while the intensity was maintained at 25 V. In separate rats (n = 5), the relationship between stimulation parameters and CBF in the somatosensory cortex was studied. In these studies, the stimulus intensity was varied between 3 and 12 V, and stimulus frequency was varied between 3 and 12 Hz.

Effect of tetrodotoxin, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-1,4-diazido[a]quinoxalin-1-one, and 7-nitroindazole on the field potentials evoked by crus II activation. In these experiments, the effect of the Na⁺ channel blocker tetrodotoxin (TTX), of the AMPA-kainate receptor inhibitor 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (NBQX), and of the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) on the field potentials produced by crus II activation was studied. The relatively selective neuronal NOS (nNOS) inhibitor 7-nitroindazole (7-NI) was also studied. TTX (10 µM) and NBQX (100 µM) were dissolved in normal Ringer. ODQ was dissolved in DMSO and diluted in Ringer (37). The final concentration of DMSO was <0.1%. 7-NI was suspended in oil and administered intraperitoneally at a dose of 50 mg/kg (n = 6). We have previously demonstrated that 7-NI at this dose attenuates NOS activity in cerebral cortex and cerebellum without affecting endothelial NOS function (18, 20). The BFcrb response to perioral stimulation was tested before and 30–45 min after administration of 7-NI or vehicle (ol, n = 5). Stimulus parameters were 5–30 V at 10 Hz for BFcrb in crus II and 3–12 V at 7 Hz for neocortical blood flow in the somatosensory cortex. The effect of 7-NI on the increase in BFcrb produced by adenosine (100 and 1,000 µM; see Ref. 37) was also tested (n = 5).

Effect of nitro-l-arginine on the increase in BFcrb evoked by crus II activation. In these experiments, we studied the effect of the nonselective NOS inhibitor nitro-l-arginine (l-NA) on the increase in BFcrb produced by crus II activation. Responses to crus II activation were tested before and 60 min after superfusion with Ringer containing l-NA (1 mM, n = 6). This concentration inhibits cerebellar NOS activity at the site of superfusion by >90% (16).

Data Analysis

Data in text, Tables 1 and 2, and Figs. 1–9 are presented as means ± SE. Comparisons between two groups were evaluated by the Student’s t-test. Multiple comparisons were evaluated by ANOVA and Tukey’s test (Systat, Evanston, IL).

RESULTS

Effect of Crus II Activation on BFcrb

Perioral stimulation (15–25 V, 1/s; n = 5) produced polysynaptic field potentials in crus II reflecting mossy fibers (P1, N1) and granule cell activation (Fig. 1A, N2; see Ref. 4). These potentials were abolished by superfusion with TTX (Fig. 1A) and could be recorded from crus II and not lobule 6. Perioral stimulation increased BFcrb in crus II (Fig. 1B; n = 6). The magnitude of the elevation depended on the intensity of stimulation and was greatest at a frequency of 10 Hz (34 ± 5%; Fig. 2). The increases in BFcrb were independent of changes in arterial pressure (Fig. 2) and were restricted to a selected portion of crus II (Fig. 1C). The increases in BFcrb produced by crus II activation, but not those elicited by hypercapnia, were abolished by superfusion.
with TTX (Fig. 1B). TTX did not affect the increase in BF$_{crb}$ produced by adenosine topically superfused at 100 µM (Ringer: 41 ± 2%; TTX: 44 ± 4%; n = 5; P > 0.05) or 1,000 µM (Ringer: 67 ± 7%; TTX: 63 ± 4%; n = 5; P > 0.05). Somatosensory stimulation produced by gently stroking the perioral region also increased BF$_{crb}$ in crus II, albeit less than electrical stimulation (18 ± 1%; P < 0.01; n = 5).

Effect of Crus II Activation on Local GU

In these experiments, we investigated whether the increases in BF$_{crb}$ produced by crus II activation were associated with increases in GU. Perioral stimulation increased GU in the ipsilateral trigeminal nucleus (+127%; P < 0.03; n = 5) and crus II (+74%; Fig. 3 and Table 2). No changes in GU (P > 0.05) were observed in the vestibular complex, reticular formation, dentate nucleus, or cerebellar vermis (Table 2).

Effect of NBQX, 7-NI, and ODQ on the Increase in BF$_{crb}$ Evoked by Crus II Activation

We then sought to define the mechanisms of the increase in BF$_{crb}$ produced by crus II activation. In agreement with previous studies (4), superfusion with the AMPA-kainate receptor antagonist NBQX attenuated the field potentials produced by perioral stimulation in crus II (Fig. 4). NBQX did not influence resting BF$_{crb}$ (Ringer: 12.9 ± 1.1 perfusion units; NBQX: 14.8 ± 0.9; P > 0.05 paired t-test; n = 5), but it attenuated the increase in BF$_{crb}$ produced by crus II activation (−71 ± 5% at 25 V; P < 0.01). However, as reported previously (37), NBQX did not attenuate significantly the increase
in BFcrb produced by hypercapnia (Ringer: 70 ± 7%; NBQX: 61 ± 11%; P > 0.05) or adenosine at 100 µM (Ringer: 39 ± 7%; NBQX: 38 ± 3%; n = 5; P > 0.05) or 1,000 µM (Ringer: 60 ± 9%; NBQX: 63 ± 6%; n = 5; P > 0.05).

To study the involvement of NO in the BFcrb response to crus II activation, we used the nNOS inhibitor 7-NI. 7-NI reduced resting BFcrb from 14 ± 1 to 11 ± 1 perfusion units (P < 0.05; n = 6). 7-NI did not influence the field potentials evoked by crus II activation, but it did reduce the increase in BFcrb by 90% (25 V; 10 Hz; P < 0.01; Fig. 5). In the same rats, 7-NI had no effect on the increase in BFcrb produced by topical application of the NO donor S-nitroso-N-acetylpenicillamine (SNAP; before 7-NI: 68 ± 8%; after 7-NI: 58 ± 5%; P > 0.05). 7-NI did not affect the increase in BFcrb produced by adenosine at 100 µM (Ringer: 41 ± 2%; 7-NI: 44 ± 4%; n = 5; P > 0.05) or 1,000 µM (Ringer: 64 ± 8%; 7-NI: 60 ± 9%; n = 5; P > 0.05).

The increase in BFcrb produced by crus II activation did not differ before and after administration of the vehicle in which 7-NI was suspended (oil; Fig. 6A; n = 5). The nonselective NOS inhibitor L-NA reduced resting BFcrb from 12 ± 1 to 10 ± 1 perfusion units (P < 0.05; n = 5) and attenuated the increase in CBF in BFcrb produced by hypercapnia (Ringer: 70 ± 7%; NBQX: 61 ± 11%; P > 0.05) or adenosine at 100 µM (Ringer: 39 ± 7%; NBQX: 38 ± 3%; n = 5; P > 0.05) or 1,000 µM (Ringer: 60 ± 9%; NBQX: 63 ± 6%; n = 5; P > 0.05).

Table 2. Effect of perioral stimulation on glucose utilization in cerebellum and brain stem

<table>
<thead>
<tr>
<th>Regions</th>
<th>Glucose Utilization, µmol·100 g⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stimulated side</td>
</tr>
<tr>
<td>RGc</td>
<td>32.8 ± 2.7</td>
</tr>
<tr>
<td>Rpc</td>
<td>34.2 ± 3.7</td>
</tr>
<tr>
<td>VC</td>
<td>96.1 ± 9.4</td>
</tr>
<tr>
<td>Sp5</td>
<td>83.8 ± 15.1*</td>
</tr>
<tr>
<td>DN</td>
<td>59.7 ± 7.1</td>
</tr>
<tr>
<td>Crus II</td>
<td>59.2 ± 7.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 experiments. Stimulation parameters: 25 V; 10 Hz; 1 s on and 1 s off; DN, dentate nucleus; RGc, nucleus reticularis gigantocellularis; Rpc, nucleus reticularis parvo-cellaris; Sp5, spinal trigeminal nucleus; VC, vestibular complex. *P < 0.03, paired t-test.

Fig. 2. Effect of stimulation parameters on the increases in BFcrb in crus II. Increases in BFcrb are related to the intensity (A) and frequency (B) of stimulation. Greatest increases in BFcrb are observed at 25 V and 10 Hz. Perioral stimulation does not influence mean arterial pressure.

Fig. 3. Increases in glucose utilization produced by perioral stimulation (25 V, 10 Hz, 1 s on and 1 s off) in brain stem and cerebellum. Stimulation increases glucose utilization in the ipsilateral trigeminal nucleus (Sp5) and in crus II (see Table 2 for values). VC, vestibular complex; PM, paramedian lobule; ICGU, local cerebral glucose utilization.
produced by crus II activation by 86% (25 V; P < 0.05; Fig. 6B). L-NA did not affect the vasodilation produced by topical application of SNAP (Ringer: 52 ± 5%; L-NA: 54 ± 6%; P > 0.05).

ODQ, a guanylyl cyclase inhibitor (10), was then used to establish whether activation of guanylyl cyclase contributes to the increase in flow. ODQ did not affect resting BFcrb (before ODQ: 13 ± 1 perfusion units; after ODQ: 12 ± 2; P > 0.05; n = 5). However, ODQ attenuated the increase in BFcrb produced by crus II activation by 63% (25 V; 10 Hz; P < 0.01) without influencing the field potentials (Fig. 7). ODQ did not affect the vasodilation produced by the cGMP-independent vasodilator adenosine topically applied at a concentration of 100 µM (vehicle: 37 ± 6%; ODQ: 37 ± 4%; n = 5; P > 0.05) or 1,000 µM (vehicle: 69 ± 6%; ODQ: 65 ± 5%; n = 5; P > 0.05).

Effect of 7-NI on the Increase in Neocortical CBF Produced by Perioral Stimulation

In these experiments, we studied the effect of 7-NI on the increase in somatosensory cortex CBF produced by perioral stimulation. We first studied the relationship between stimulation parameters and CBF response. The greatest increases in CBF were obtained at 10 V and 7 Hz (Fig. 8; n = 5). We then investigated the effect of 7-NI on the CBF response. 7-NI reduced resting CBF from 12 ± 1 to 10 ± 1 perfusion units (n = 5; P < 0.05) and attenuated the increase in CBF produced by perioral stimulation by 52% (10 V; 7 Hz; P < 0.01; Fig. 9).

DISCUSSION

Novel Findings of the Study and Relationship to Previous Investigations

We sought to investigate further the mechanisms of the local regulation of the cerebellar microcirculation during neural activity. Although it is well known that neural activity is a major determinant of CBF, most investigations have focused on the cerebral cortex, and less attention has been paid to the neural mechanisms regulating the cerebellar microcirculation (see Ref. 17 for a review). Previous investigations have used activation of the PF or CF inputs to Purkinje neurons as a model to study the relationship between synaptic activ-

Fig. 4. Effect of 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-kainate receptor inhibitor 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-[f]isoxazolines (NBQX) on field potentials (A) and BFcrb increases (B) in crus II during perioral stimulation. NBQX attenuates the field potentials and the BFcrb elevations produced by crus II activation.

Fig. 5. Effect of neuronal (n) nitric oxide synthase (NOS) inhibitor 7-nitroindazole (7-NI) on the field potentials (A) and BFcrb increases (B) in crus II during perioral stimulation. 7-NI does not attenuate the field potentials produced by perioral stimulation, but it substantially reduces the BFcrb elevations.
ity and BFcrb (1, 16, 17, 19, 36, 37). Although these studies have provided an insight into the mechanisms regulating BFcrb during neural activation, stimulation of individual inputs to Purkinje cells is unlikely to reflect accurately the physiological pattern of neural activity that occurs during “natural” cerebellar function. In this study, therefore, we developed a new model of functional hyperemia in cerebellar cortex based on activation of somatosensory inputs to the cerebellum. Specifically, we examined the changes in BFcrb produced by activation of crus II, an area of the cerebellar cortex that receives sensory afferents from the region of the face (30). We found that electrical or mechanical stimulation of the perioral region increases BFcrb in crus II. The increases depend on the intensity and frequency of the stimulus, are blocked by TTX, and are associated with focal elevations in GU. These observations suggest that the increases in BFcrb are related to increases in local synaptic activity and energy metabolism.

We then investigated the transmitters and vascular mediators involved in the increase in BFcrb. We found that inhibition of AMPA-kainate receptors by NBQX attenuates the field potentials and reduces the increase in BFcrb produced by crus II activation. To determine whether NO, a potent vasodilator released by activation of glutamate receptors, is involved in the vascular response, the relatively selective nNOS inhibitor 7-NI was used. It was found that 7-NI nearly blocks the increase in BFcrb without affecting the field potentials evoked by crus II activation. In contrast, in somatosensory cortex, 7-NI attenuated the CBF response to perioral stimulation only by ≤50%. NO produces vasodilation, mainly by activating soluble guanylyl cyclase (see Ref. 7 for a review). To determine whether the vasodilation is dependent on soluble guanylyl cyclase activation, we used the inhibitor ODQ (10). We found that ODQ attenuates the increase in BFcrb without affecting the field potentials evoked by crus II activa-
These data, collectively, indicate that the increases in BF\textsubscript{crb} initiated by activation of crus II are mediated by activation of glutamate receptors via production of NO and cGMP.

Exclusion of Potential Sources of Artifacts

The increases in BF\textsubscript{crb} produced by crus II activation cannot be a consequence of changes in arterial pressure or blood gases because these parameters were closely monitored and did not differ among groups. Similarly, the elevation in BF\textsubscript{crb} elicited by perioral stimulation is not secondary to a global arousal reaction because the changes in flow and GU were not diffuse but were restricted to a subregion of crus II. Furthermore, the attenuation of the response to crus II activation by NBQX, ODQ, and 7-NI cannot be attributed to nonspecific effects of these agents, resulting in vasoparalysis. NBQX, while attenuating the flow response to crus II activation, did not affect the vasodilation produced by hypercapnia or adenosine. ODQ attenuated the response to crus II activation but not the increase in BF\textsubscript{crb} produced by the cGMP-independent vasodilator adenosine, whereas 7-NI and L-NA blocked the response to crus II activation but did not affect the vasodilation produced by the NO donor SNAP or adenosine. The effect of 7-NI is unlikely to result from inhibition of other isoforms of NOS. Whereas the inducible or "immunological" isoform of NOS is not expressed in the intact cerebellum (27), we have previously demonstrated that the endothelial isoform of NOS is not inhibited by 7-NI in this preparation (18). The relatively high concentration of L-NA used in this study (1 mM) was needed to assure penetration of the drug in sufficient concentrations to inhibit NOS in the cerebellar parenchyma. Such concentration of L-NA virtually abolishes cerebellar NOS activity in the field of superfusion (16). However, 1 mM L-NA does not have nonspecific vascular effects in this preparation (16). The possibility has been raised that L-NA also inhibits ATP-dependent K\textsuperscript{+} channels (23). However, in our preparation, the effect of L-NA is unlikely to be due to inhibition of K\textsuperscript{+} channels rather than NOS, because 7-NI, an NOS inhibitor structurally unrelated to L-NA, and ODQ, a guanylyl cyclase inhibitor, also attenuate the response. The reproducibility in time of the BF\textsubscript{crb} response to crus II activation is demonstrated by the fact that flow increases were not different when tested before and 30–45 min after administration of the vehicle for 7-NI. Therefore, the findings of the present study cannot be attributed to cerebrovascular actions of systemic variables, instability of the preparation, or nonspecific vascular effects of the pharmacological agents used.

Fig. 8. Effect of stimulation parameters on increases in somatosensory cortex CBF. Increases in CBF are related to intensity (A) and frequency (B) of stimulation and are greatest at 10 V and 7 Hz.

Fig. 9. Effect of nNOS inhibitor 7-NI on CBF increases in somatosensory cortex during perioral stimulation. 7-NI reduces the BF\textsubscript{crb} elevations by \textasciitilde 50%.

\[ *p<0.01 \]
Neural Mechanisms of the Increases in BF\textsubscript{crb}

Perioral stimulation activates trigeminal afferents that reach crus II via the mossy fibers-granule cell-PF pathway and the inferior olive-CF pathway (28, 34). The observation that the field potentials and BF\textsubscript{crb} increases are attenuated by the AMPA-kainate receptor inhibitor NBQX is consistent with the hypothesis that the response depends on glutamate release and AMPA-kainate receptor activation in crus II. The present study did not address the question of whether N-methyl-D-aspartate (NMDA) receptors are involved in the electrophysiological and hemodynamic response evoked by crus II activation. However, previous investigations suggest that NMDA receptors do not participate in the hemodynamic responses evoked by activation of the PF or the CF (1, 37). Because these pathways are both activated during perioral stimulation, it is conceivable that NMDA receptors do not contribute to the response. However, this critical issue needs to be addressed in future experiments in which the specific glutamate receptor subtypes mediating the hemodynamic changes are identified.

Because glutamate is not vasoactive (6), it is unlikely that this transmitter is directly responsible for relaxation of vascular smooth muscles and vasodilation. The observation that the increase in BF\textsubscript{crb} is inhibited markedly by the nNOS inhibitor 7-NI and by the guanylyl cyclase inhibitor ODQ suggests that AMPA-kainate receptor activation elicits NO production and that, in turn, NO produces vasodilation by activating soluble guanylyl cyclase. However, it is surprising that ODQ attenuates the flow response less than 7-NI. A possible explanation for this finding is that superfusion with ODQ (100 \mu M) did not completely block guanylyl cyclase activity in our preparation. In support of this hypothesis is the observation that ODQ, at the doses used in the present study, does not completely block responses to guanylyl activation by SNAP in the rat cerebellum (37). Similarly, ODQ substantially attenuates, but does not block, the vasodilation produced by the NO-generating agents nitroprusside or ACh (8). On the other hand, NO could produce vasodilation by activating soluble guanylyl cyclase; however, it is surprising that ODQ attenuates the flow response less than 7-NI. A possible explanation for this finding is that superfusion with ODQ (100 \mu M) did not completely block guanylyl cyclase activity in our preparation. In support of this hypothesis is the observation that ODQ, at the doses used in the present study, does not completely block responses to guanylyl activation by SNAP in the rat cerebellum (37). Similarly, ODQ substantially attenuates, but does not block, the vasodilation produced by the NO-generating agents nitroprusside or ACh (8). On the other hand, NO could produce vasodilation by activating soluble guanylyl cyclase; however, it is surprising that ODQ attenuates the flow response less than 7-NI.

Role of NO in the Response to Crus II Activation

The cellular sources of NO during crus II activation have not been established and cannot be defined on the basis of the results of the present study. In the cerebellar molecular layer of rodents, NOS is present mainly in interneurons and stellate and basket cells but not in Purkinje cells (3, 22, 33). It is therefore likely that, during crus II activation, NO is produced by stellate and basket cells activated by inputs from CF and PF. There is evidence that molecular layer interneurons may be an important source of NO during PF stimulation. Stimulation of the CF in transgenic mice with targeted disruption of Purkinje cells (P03 mice) produces increases in BF\textsubscript{crb} smaller than those observed in nontransgenic littermates (36). However, at variance with intact mice, the residual component of the BF\textsubscript{crb} increase is entirely dependent on NO (36). Considering that molecular layer interneurons are morphologically intact in P03 mice (36), it is most likely that stellate and/or basket cells are the source of NO. On the other hand, NO could also be released directly from PF terminals (24, 31). In one study in which NO-sensitive electrodes were used, NO production from PF could not be attenuated by AMPA-kainate receptor inhibitors (31). Because in the present study the BF\textsubscript{crb} flow increase could be attenuated substantially by AMPA-kainate receptor inhibition, it is unlikely that the PF are the major source of NO during somatosensory activation. However, this possibility needs to be tested experimentally.

Role of NO in the Response to Somatosensory Activation in Neocortex.

Most studies of functional hyperemia have focused on the cerebral cortex, and very little is known about the mechanisms regulating blood flow during synaptic activity in other brain regions. Using a model of somatosensory activation that increases flow both in cerebral cortex and cerebellar cortex, we were able to study the role of NO in the vascular response to...
synaptic activity in these two regions. We found that, under identical experimental conditions, NOS inhibition by 7-NI attenuates the increase in flow produced by somatosensory stimulation more in cerebellar cortex than in cerebral cortex. We have previously demonstrated that 7-NI (50 mg/kg ip) produces comparable degrees of NOS inhibition in cerebellum (≈60%; see Ref. 18) and cerebral cortex (≈70%; see Ref. 20). The finding that NOS inhibition affects functional hyperemia more in cerebellum than in neocortex has two major implications. First, it provides evidence that the mechanisms responsible for coupling neural activity to blood flow are regionally specific. Thus the role that vasoactive neurotransmitters and neuromodulators play in functional hyperemia depends on the brain region. Second, it suggests that, during somatosensory activation, NO plays a more prominent role in the regulation of flow in cerebellum than in cerebral cortex. This is not surprising considering that NO is most abundant in cerebellum and plays a critical role in cerebellar synaptic function (5). As for the involvement of glutamate receptors in the mechanisms of functional hyperemia in neocortex, preliminary data suggest that both NMDA and non-NMDA receptors are involved in the vasodilation produced by sciatic nerve stimulation in the rat somatosensory cortex (26). Therefore, in neocortex as in cerebellum, glutamate receptors play a prominent role in the mechanisms of functional hyperemia.

Conclusions

We have investigated the mechanisms of functional hyperemia in cerebellar cortex using activation of crus II by somatosensory stimulation as a model. We found that stimulation of trigeminal afferents from the region of the face increases BFcrb in crus II. The increases in flow are dependent on local synaptic activity, are associated with increased GU, and are attenuated by inhibition of AMPA-kainate receptors. Furthermore, the flow response is virtually abolished by nNOS inhibition and markedly attenuated by inhibition of soluble guanylyl cyclase. In contrast, the increase in CBF produced by the same somatosensory stimulus in cerebral cortex is reduced only by ≈50% by NOS inhibition. Although the evidence suggests that NO plays a prominent role in the mechanisms of coupling synaptic activity to blood flow in the cerebellar cortex, it also indicates that the mechanisms regulating cerebral perfusion during synaptic activity are regionally distinct.

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