Effect of adenosine receptor blockade on pial arteriolar dilation during sciatic nerve stimulation

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Received 22 March 2001; accepted in final form 18 July 2001

Meno, Joseph R., Alison V. Crum, and H. Richard Winn. Effect of adenosine receptor blockade on pial arteriolar dilation during sciatic nerve stimulation. Am J Physiol Heart Circ Physiol 281: H2018–H2027, 2001.—In the present study, we report the effects of adenosine receptor antagonists on pial vasodilatation during contralateral sciatic nerve stimulation (SNS). The pial circulation was observed through a closed cranial window in α-chloralose-anesthetized rats. In artificial cerebrospinal fluid (CSF), SNS resulted in a 30.5 ± 13.2% increase in pial arteriolar diameter in the hindlimb somatosensory cortex. Systemic administration of the selective adenosine A2A receptor antagonist, 4-([2-([7-amino-2-[2-furyl]]triazolol[2,3-a][1,3,5]triazin-5-yl-amino}ethyl)phenol (ZM-241385), significantly (P < 0.05, n = 6) attenuated the SNS-induced vasodilatation. Systemic administration of 8-(p-sulfophenyl)theophylline (8SPT), a nonselective antagonist that is blood-brain barrier (BBB) impermeable, had no effect on vasodilatation to SNS. In contrast, systemic theophylline, which readily penetrates the BBB, nearly abolished the SNS-induced vasodilatation (P < 0.01; n = 7). Topical superfusion of 8SPT significantly (P < 0.01; n = 6) attenuated vasodilatation during SNS. Topical superfusion of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective adenosine A1 receptor antagonist, significantly potentiated SNS-induced vasodilatation (P < 0.01; n ≥ 5). Hypercarbic vasodilatation and somatosensory-evoked potentials were not affected by any of the compounds tested. Our findings suggest that luminal endothelial adenosine receptors are not involved in the arteriolar response to SNS, as demonstrated by a lack of effect with systemic 8SPT. Furthermore, the adenosine A2A receptor subtype appears to be involved in the dilator response to SNS. Finally, the neuromodulatory action of adenosine, via the A1 receptor subtype, significantly influences SNS-induced vasodilatation. Thus the present study provides further evidence for a role of adenosine in the regulation of CBF during somatosensory stimulation.

ZM-241385; DPCPX; 8SPT; theophylline; EHNA; pial circulation; neuronal activity

PIAL ARTERIOLES IN THE HINDLIMB SOMATOSENSORY CORTEX dilate in a characteristic manner in response to contralateral sciatic nerve stimulation (SNS) (32). The SNS-induced changes in pial arteriolar diameter closely parallel changes in cerebral blood flow (CBF), as measured by laser Doppler flowmetry (35). Adenosine, a breakdown product of ATP, is a proposed regulator of CBF under a variety of conditions, including during increased cortical activity (8, 22, 23). A study by Ko et al. (22) from our laboratory, reported that topical application of theophylline (Theo), a nonselective adenosine receptor antagonist, significantly attenuated the pial arteriolar response to SNS. These investigators also reported that dipyridamole or inosine, which compete with adenosine for uptake, potentiated the vascular response to SNS. Other studies have confirmed the observations by Ko et al. (22). For example, Dirnagl et al. (8) demonstrated that either Theo or adenosine deaminase significantly attenuated the increases in laser Doppler flow during whisker stimulation. Furthermore, Li and Iadecola (23) reported attenuation of CBF in cerebellar cortices by Theo during neuronal activation. Thus these studies support the contention that adenosine is involved in the regulation of CBF during increased cortical activity, but little information exists as to the involved adenosine mechanisms and receptors. However, the role of adenosine in the regulation of CBF during increased cortical activity is not without controversy. With the use of a modified microdialysis cannula equipped with a hydrogen clearance electrode, Northington et al. (38) were unable to affect the CBF response to SNS using 8SPT. Gotth et al. (14), employing an autoradiographic technique (4-[N-methyl-14C]jodoantipyrine; [14C]IAP) to measure CBF, recently noted in unanesthetized rats that caffeine only attenuated CBF statistically in trigeminal nuclei during neuronal activation evoked by whisker-barrel stimulation but was without an attenuating effect in the thalamus and cortex. In fact, in the latter location (i.e., barrel cortex), these investigators noted a suggestion of hyperemia.

Adenosine has many of the prerequisites necessary to participate in the coupling between local CBF and brain metabolism. For example, adenosine is a potent dilator of pial arterioles in vivo (19, 28), and of cerebral intraparenchymal arterioles in vitro (10, 15, 37). Adenosine is also a potent inhibitor of neuronal transmission (13, 44). Thus, in addition to increasing blood supply, adenosine is capable of decreasing metabolic...
The actions of adenosine are mediated through extracellular cell surface receptors and cloning experiments have identified four adenosine receptor subtypes in the brain: A1, A2A, A2B, and A3 (9, 12). The A1 receptors act both presynaptically to inhibit neurotransmitter release (13) and postsynaptically to inhibit neuronal firing (44). In general, A1 receptor activation is much less effective at inhibiting transmitter release and neuronal excitability of inhibitory neurons. Thus activation of A1 receptors would tend to reduce CNS activity and blockade of the A1 receptor would tend to increase CNS activity. The A2 receptor subtype is further subdivided into A2A and A2B. A2A (6, 18) causes relaxation of vascular smooth muscle leading to vasodilation and increased blood flow. The A2B receptor subtype may also play a role in cerebral vasodilation. However, lack of selective A2B agonists or antagonists has hampered studies attempting to determine the role of the A2B receptor subtype in CBF regulation. The function of the A3 receptor subtype in the cerebral circulation is also poorly defined. The A3 adenosine receptor subtype appears to be located in the hippocampus and on astrocytes, however, its physiological function is unclear, in part, related to the absence of selective agonists and antagonists.

Adenosine-induced vasodilation in many vascular beds is well documented (5, 10, 15). In a study of intracerebral arterioles in vitro, Ngai and Winn (37) reported potent dose-dependent dilation to extraluminal application of adenosine. However, intraluminally applied adenosine causes vasodilation by first diffusing into the extraluminal space to reach adenosine receptors (37). Thus, in intracerebral arterioles, adenosine receptors associated with vasodilation do not appear to be present on the luminal surface of vascular endothelium (37). In contrast to the cerebral circulation, the coronary endothelium appears to play a significant role in adenosine-induced vasodilation (30). Hein et al. (18) reported that denudation of coronary arterioles significantly attenuated adenosine- and 2-(2-carboxyethyl)-phenethylamino-5′-N-ethylcarboxyamidoadenosine (CGS-21680)-induced dilations. These findings suggest that the role of the endothelium may vary among the different vascular beds and depending on the physiological stimulus. The role of the endothelium or endothelial adenosine receptors in CBF regulation during somatosensory stimulation is currently unknown.

In the present study, we investigated the effect of adenosine receptor antagonists on the pial arteriolar response to SNS. We report 1) the effect of adenosine A2A receptor antagonist with the use of 4-[2-[(7-aminooxy-2-[2-furyl][3,2,4](triazolol[2,3-a][1,3,5]triazin-5-yl-amino)ethyl]phenol (ZM-241385), 2) the effect of adenosine A1 receptor antagonist with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 3) the effect of intraluminal versus extraluminal adenosine receptor blockade utilizing 8-(p-sulphophenyl)theophylline (8SPT) and Theo, and 4) the effect of blockade of adenosine deaminase with erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). We hypothesized that A2A antagonism would significantly reduce vasodilatation during SNS, whereas A1 blockade with DPCPX would potentiate the response. We further hypothesize that systemically administered 8SPT would have no effect on the pial vascular response to SNS whereas topical application would attenuate the response. On the other hand, systemic administration of the permeable antagonist Theo would significantly reduce the SNS-induced vasodilatation.

METHODS

Animal preparation. The experimental protocols in the present study were approved by the University of Washington Animal Welfare Committee. Male Sprague-Dawley rats weighing 350–400 g were initially anesthetized with halothane (1.5–2.0%). The right femoral artery was cannulated to allow monitoring of blood pressure and blood gas sampling. The right femoral vein was also cannulated for intravenous drug administration. The rats were tracheotomized, immobilized with the use of tubocurarine chloride (1 mg/kg iv), and mechanically ventilated. The ventilated gases were adjusted to maintain physiological blood gas tensions, and rectal temperature was maintained at 37°C. Halothane anesthesia was discontinued and replaced with α-chloralose and urethane (50 and 500 mg/kg ip, respectively). The animals were positioned on a stereotaxic frame and the pial vasculature over the right somatosensory cortex was visualized through a closed cranial window as previously described (28, 32).

The contralateral sciatic nerve was exposed, cut proximal to the bifurcation into tibial and peroneal nerves, and placed on stimulating electrodes that were immersed in a pool of warm mineral oil. An automatic video dimension analyzer (model 303, IPM; San Diego, CA), which tracks the width of red blood cell columns, allowed continuous on-line monitoring of pial vessel diameter. The contralateral sciatic nerve was stimulated for 20 s (0.20 V, 5 Hz, 0.5 ms rectangular pulses) and vascular response to SNS was calculated as a percent increase over resting diameter. A study by Ngai et al. (32) revealed that these stimulation parameters optimize the vascular response without affecting systemic blood pressure.

Adenosine, Theo, 8SPT, DPCPX, CGS-21680, and EHNA were obtained from Research Biochemicals International (Natick, MA). ZM-241385 was purchased from Toecis (Baldwin, MO). ZM-241385 was initially dissolve in NaOH-polyethylene glycol 400 (50:50, vol/vol) and then diluted in saline before administration. Theo and 8SPT (40 mmol/l) were directly dissolved in isotonic saline. Stock solutions of 8SPT, CGS-21680, and adenosine were prepared in ultrafiltered water and then diluted in artificial cerebrospinal fluid (CSF) to reach final desired concentrations for topical application. A stock solution of DPCPX (10 mM; 50% ethanol-50% alkamuls (Rhone Poulenc; Cranbury, NJ)) was made in advance and subsequently diluted in artificial CSF to arrive at the desired final concentration. Vehicle concentration (ethanol-alkamuls) was <0.005% in all of the concentrations tested. Vehicle control studies for ZM-241385 or DPCPX revealed no effect of the vehicle on resting arteriolar diameter, hypercarbic vasodilation, or SNS-induced vasodilatation. The artificial CSF was composed of (in meq/l) 156.5 Na+, 2.95 K+, 2.50 Ca2+, 1.33 Mg2+, 24.6 HCO3-, 66.5 dextrose, and 40.2 urea.
Preliminary experiments were performed to determine the concentration of 8SPT in plasma and CSF after systemic administration (0.05 μmol/g ip). CSS samples were collected from a closed cranial window as previously described (26) and blood samples were obtained via a femoral artery catheter. Plasma and CSF samples were analyzed for 8SPT with the use of reverse-phase high-performance liquid chromatography (16). Retention times and peak heights of a known standard were used to identify and calculate the concentration of 8SPT in each sample.

Experimental protocol. Artificial CSF was superfused under the cranial window for a minimum of 10 min, followed by a 1- to 2-min period to allow vessel diameter and intracranial pressure to stabilize before stimulation of the contralateral sciatic nerve. The sciotic nerve was stimulated for 20 s and changes in vessel diameter were recorded. This protocol was repeated with CSF containing 8SPT, DPCPX, or EHNA. Only one compound was tested per animal, except in those animals where the effect of Theo on EHNA-induced potentiation was evaluated. A minimum of three acquisitions per test compound was performed. Thus, over the course of the experimental protocol, each test compound was topically applied for a minimum of 30 min. Separate experiments were performed to determine the effects of systemic administration of ZM-241385, 8SPT, or Theo at 30-min postinjection. Mean arterial blood pressure (MABP) and arterial PCO2 and PO2 (PaCO2, and PaO2), respectively, were monitored and remained stable throughout the entire duration of the experiment. In addition, reactivity of pial arterioles to CO2 inhalation was assessed before and after exposure to the test compounds, as previously described (28). Briefly, the respirator was switched to a special gas mixture containing 6% CO2-10% O2-84% N2 for 2 min. Percent increase in pial arteriolar diameter was calculated and CO2 reactivity expressed as a percent increase in diameter per millimeters of mercury increase in PaCO2. The pial arteriolar response to CO2 inhalation was not affected by any of the compounds tested in this study.

In separate groups of animals, somatosensory-evoked potentials (SSEPs) were monitored during SNS with an electrodagnostic monitoring system (Biologic Systems; Mundelein, IL). The sciotic nerve was stimulated with constant current rectangular pulses, with the stimulation parameters identical to those utilized for studying the vascular response (0.5-ms pulses, 5 Hz, 20 s). Consecutive SSEPs were averaged with filtering set to a bandwidth from 3 to 3,000 Hz. In these experiments, the cranial window was left open but superfused continuously at 0.1 ml/min with artificial CSF. Only one test compound was tested per animal. CSF containing a test compound was superfused for 15 min. Superfusion was then stopped and excess CSF was removed. SSEPs were recorded with a silver ball-tip electrode placed on the hindlimb somatosensory cortex by means of a micromanipulator. Evoked potentials were recorded 30-min postinjection for systemically administered compounds. SSEP acquisitions were 5 min apart and a minimum of five acquisitions was made per test compound. Thus each test compound was administered for >30 min over the course of the experiment. A time-control study for SSEPs utilizing this technique has previously been reported (35).

Statistical analysis. All values are expressed as means ± SD. Analysis of variance and Student’s paired t-tests were used to make statistical comparisons. When multiple simultaneous comparisons were made, a Bonferroni correction was applied. A P value of <0.05 was used to indicate statistically significant differences.

RESULTS

Systemic administration of 8SPT and Theo. The concentration of Theo in both plasma and CSF has previously been shown to be significantly elevated 30 min after intraperitoneal administration (29). In contrast, the pharmacokinetics of 8SPT has not previously been reported. Therefore, our initial experiments were aimed at determining the concentrations of 8SPT in plasma and CSF after intraperitoneal administration. This group of animals received an intraperitoneal injection of 8SPT (0.05 μmol/g). CSF and plasma samples were collected at 10, 20, and 30 min postadministration. The plasma concentration (Table 2) of 8SPT was 26.2 ± 4.5 μmol/l 10 min after intraperitoneal injection and remained elevated at 30-min postinjection (23.0 ± 4.1 μmol/l). These levels of 8SPT are similar to previously reported plasma Theo levels with the same dose and route of administration (29). In contrast with the plasma levels, CSF concentrations of 8SPT (Table 2) were relatively unchanged following systemic administration of 8SPT.

Before 8SPT was administered, pial arterioles dilated 25.9 ± 6.0% during SNS (n = 7). Systemic administration of 8SPT (0.05 μmol/g ip) had no effect on resting diameter, MABP, PaCO2, or PaO2 (Table 1). Moreover, this dose of 8SPT had no effect on pial vasodilation during SNS (Fig. 1). In contrast to 8SPT, Theo (0.05 μmol/g ip; 30 min) almost completely abolished the vascular response to SNS (Fig. 1; P < 0.01, n = 7). Thus intraperitoneal administration of Theo was more effective at attenuating the vasodilatation in response to SNS than topical application (22). PaO2 and MABP were stable and within physiological range (Table 1).

Systemic administration of 8SPT or Theo had no effect on reactivity of pial arterioles to CO2 inhalation (Control, 1.41 ± 0.48%/mmHg; 8SPT, 1.39 ± 0.50%/mmHg; Theo, 1.41 ± 0.51%/mmHg, n = 5). Theo also had no effect on SSEPs. During control conditions and before Theo administration, the amplitude of the N1 and P1 waves were −1,455.0 ± 430.6 and 927.8 ± 242.6, respectively. Thirty minutes after intraperitoneal administration of Theo, the N1 and P1 waves were −1,025.6 ± 269.0 and 847.6 ± 369.0, respectively. Topical application of 8SPT. In artificial CSF, stimulation of the contralateral sciatic nerve resulted in a 26.4 ± 4.6% increase in pial arteriolar diameter in the hindlimb sensory cortex (n = 6). Topically applied 8SPT had no effect on MABP, PaCO2, PaO2, or resting arteriolar diameter (Table 1). Three different concentrations of 8SPT were tested: 0.1, 1, and 10 μmol/l. The pial circulation was exposed to each concentration of 8SPT for a minimum of 30 min. A downward trend was observed in the pial vasodilation during SNS with 0.1 μmol/l 8SPT. In contrast, with topical application of 1 or 10 μmol/l of 8SPT, significant attenuation of the SNS-induced pial vasodilation occurred (Fig. 2, P < 0.01; n = 6) compared with control. The inhibitory effect of 8SPT was reversed after washout of the subwindow space with artificial CSF. PaO2, PaCO2,
Table 1. Physiological parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline Diameter, μm</th>
<th>MABP, mmHg</th>
<th>Arterial pH</th>
<th>PaCO₂, mmHg</th>
<th>PaO₂, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.6 ± 3.0</td>
<td>119.4 ± 17.3</td>
<td>7.37 ± 0.03</td>
<td>37.1 ± 4.7</td>
<td>116.1 ± 28.5</td>
</tr>
<tr>
<td>SSPT (10⁻⁷ M)</td>
<td>31.4 ± 2.7</td>
<td>116.0 ± 16.0</td>
<td>7.37 ± 0.02</td>
<td>38.4 ± 4.2</td>
<td>110.9 ± 21.8</td>
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<tr>
<td>SSPT (10⁻⁶ M)</td>
<td>31.4 ± 4.0</td>
<td>121.1 ± 14.2</td>
<td>7.37 ± 0.03</td>
<td>37.9 ± 4.7</td>
<td>119.4 ± 24.7</td>
</tr>
<tr>
<td>SSPT (10⁻⁵ M)</td>
<td>31.9 ± 2.7</td>
<td>116.0 ± 13.1</td>
<td>7.39 ± 0.03</td>
<td>35.9 ± 3.8</td>
<td>123.0 ± 27.3</td>
</tr>
<tr>
<td>Control</td>
<td>27.5 ± 7.2</td>
<td>123.3 ± 9.7</td>
<td>7.38 ± 0.05</td>
<td>33.0 ± 4.6</td>
<td>111.6 ± 16.1</td>
</tr>
<tr>
<td>ZM-241385 (1 mg/kg iv)</td>
<td>25.7 ± 7.4</td>
<td>124.7 ± 9.7</td>
<td>7.38 ± 0.04</td>
<td>33.2 ± 2.7</td>
<td>111.7 ± 16.5</td>
</tr>
<tr>
<td>ZM-241385 (5 mg/kg iv)</td>
<td>30.2 ± 6.0</td>
<td>120.0 ± 9.9</td>
<td>7.38 ± 0.06</td>
<td>33.6 ± 2.4</td>
<td>114.0 ± 17.3</td>
</tr>
<tr>
<td>Control</td>
<td>31.2 ± 3.3</td>
<td>104.0 ± 5.2</td>
<td>7.36 ± 0.03</td>
<td>34.3 ± 2.4</td>
<td>107.4 ± 4.8</td>
</tr>
<tr>
<td>DPCPX (10⁻⁸ M)</td>
<td>30.4 ± 2.4</td>
<td>104.4 ± 9.5</td>
<td>7.35 ± 0.02</td>
<td>34.0 ± 2.1</td>
<td>105.6 ± 5.7</td>
</tr>
<tr>
<td>DPCPX (10⁻⁷ M)</td>
<td>30.1 ± 2.1</td>
<td>107.6 ± 9.9</td>
<td>7.35 ± 0.02</td>
<td>33.7 ± 1.9</td>
<td>106.0 ± 8.5</td>
</tr>
<tr>
<td>DPCPX (10⁻⁶ M)</td>
<td>30.5 ± 2.3</td>
<td>107.2 ± 10.4</td>
<td>7.34 ± 0.02</td>
<td>34.7 ± 2.9</td>
<td>106.2 ± 10.9</td>
</tr>
<tr>
<td>Control</td>
<td>30.6 ± 3.5</td>
<td>118.5 ± 6.9</td>
<td>7.38 ± 0.02</td>
<td>35.6 ± 2.4</td>
<td>127.1 ± 23.6</td>
</tr>
<tr>
<td>SSPT (0.05 μmol/g ip)</td>
<td>30.6 ± 3.4</td>
<td>120.4 ± 9.9</td>
<td>7.37 ± 0.03</td>
<td>36.7 ± 2.8</td>
<td>137.9 ± 26.3</td>
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<tr>
<td>Theo (0.05 μmol/g ip)</td>
<td>30.5 ± 3.3</td>
<td>120.4 ± 9.9</td>
<td>7.38 ± 0.02</td>
<td>36.9 ± 2.6</td>
<td>129.0 ± 14.2</td>
</tr>
<tr>
<td>Control</td>
<td>32.4 ± 2.0</td>
<td>115.8 ± 11.9</td>
<td>7.44 ± 0.03</td>
<td>35.8 ± 2.9</td>
<td>113.6 ± 15.8</td>
</tr>
<tr>
<td>EHNA (10⁻⁶ M)</td>
<td>31.8 ± 2.2</td>
<td>114.3 ± 10.1</td>
<td>7.41 ± 0.04</td>
<td>34.7 ± 4.0</td>
<td>108.4 ± 12.1</td>
</tr>
<tr>
<td>EHNA (10⁻⁵ M)</td>
<td>32.5 ± 1.9</td>
<td>118.6 ± 11.5</td>
<td>7.42 ± 0.03</td>
<td>36.7 ± 3.0</td>
<td>111.8 ± 16.7</td>
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</table>

Values are means ± SD. MABP, mean arterial blood pressure; PaCO₂, arterial P CO₂; PaO₂, arterial P O₂; SSPT, 8-(β-sulfophenyl)theophylline; ZM-241385, 4(R)-2-[7 amino-2-[2-furyl][3,2,4]triazolol[2,3-a][1,3,5]triazin-5-ylamino]ethyl) phenol; Theo, theophylline; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EHNA, erythro-9-(2-hydroxy-3-nonyl) adenine.

MABP were stable and within physiological range during SNS and no significant differences were observed between the experimental groups.

Topical application of SSPT had no effect on pial arteriolar dilation to CO₂ inhalation (Control, 1.33 ± 0.37%/mmHg; SSPT, 1.39 ± 0.23%/mmHg). Topical application also had no effect on SSEPs. Before superfusion of SSPT, the amplitude of the N₁ and P₁ waves, were −1.507.2 ± 340.0 and 985.7 ± 223.9, respectively. After topical application of SSPT, N₁ and N₂ waves, were −1.430.2 ± 218.0 and 1,021.7 ± 346.2, respectively.

**Systemic and topical administration of ZM-241385.** Stimulation of the contralateral sciatic nerve in this group of animals (n = 6) induced a 30.5 ± 13.2% increase in pial arteriolar diameter under control conditions. Systemic administration of ZM-241385 (0.1, 1, or 5 mg/kg iv; 30 min) had no effect on resting arteriolar diameter. Moreover, ZM-241385 administration at any of the doses tested had no effect on MABP or PaO₂ and PaCO₂ (Table 1).

The data for the effects of three different doses of ZM-241385 during SNS are presented in Fig. 3. During SNS, the lowest dose of ZM-241385 (0.1 mg/kg) had no effect on pial arteriolar dilation. However, after a dose of 1 mg/kg, the SNS-induced vasodilatation was significantly reduced by −50% (n = 6, P < 0.05). Moreover, 30 min after an intravenous injection of ZM-241385 (5 mg/kg), the SNS-induced vasodilatation was reduced by −80% (P < 0.05). In contrast to systemic administration, topical application of ZM-241385 (0.01 to 1 μmol/l) had no effect on pial vasodilatation during SNS (data not shown) paralleling the similarly less potent effect of topical Theo compared with systemic Theo.

In separate experiments, we tested the effect of systemic administration of ZM-241385 (1 mg/kg iv) on pial arteriolar dilation to topical application of the A₂A selective agonist CGS-21680 (10 nmol/l) or adenosine (10 μmol/l). These results are presented in Fig. 4. In brief, topical application of either CGS-21680 or adenosine induced significant vasodilatation of pial arterioles, which was significantly attenuated by systemically administered ZM-241385 (1 mg/kg iv; P < 0.01; n = 5). We also tested the effects of topical application

Table 2. Concentration of SSPT after intraperitoneal administration

<table>
<thead>
<tr>
<th></th>
<th>Plasma concentrations, μmol/l</th>
<th>CSF concentrations, μmol/l</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n 10 min 20 min 30 min</td>
<td>n 10 min 20 min 30 min</td>
</tr>
<tr>
<td>Plasma</td>
<td>5 26.2 ± 10.0 24.3 ± 11.6 23.0 ± 9.2</td>
<td>4 0.05 ± 0.04 0.09 ± 0.02 0.09 ± 0.03</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
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</table>

Values are means ± SD; n, no. of rats. CSF, cerebrospinal fluid. This group of animals received an intraperitoneal injection of 0.05 μmol/g SSPT.

Fig. 1. Effect of systemically administered 8-(β-sulfophenyl)theophylline (SSPT; 0.05 μmol/g ip) and theophylline (Theo; 0.05 μmol/g ip) on pial arteriolar dilation during sciatic nerve stimulation. All values are means ± SD, n = 7; *P < 0.01 vs. Control.
of ZM-241385 on pial arteriolar dilation to topical application of CGS-21680 (10 nmol/l) or adenosine (10 μmol/l). In this group of animals, topical application of CGS-21680 or adenosine induced pial arteriolar dilations of 27.45 ± 6.79% and 17.03 ± 2.64%, respectively. In the presence of ZM-241385 (1 μmol/l), dilations to CGS-21680 or adenosine were 4.03 ± 1.66 and 5.43 ± 2.47%, respectively (P < 0.05, n = 5; *P < 0.01 vs. control).

Intravenous administration of ZM-241385 had no effect on hypercarbic vasodilatation. Under control conditions, CO₂ reactivity was 1.75 ± 0.23%/mmHg. After intravenous administration of ZM-241385, CO₂ reactivity was 1.81 ± 0.64%/mmHg (n = 4). ZM-241385 also had no effect on SSEPs. Before administration of ZM-241385, the amplitude of the N1 and P1 waves were 2,114.1 ± 291.9 and 1,120.3 ± 379.6, respectively. Thirty minutes after intraperitoneal administration of ZM-241385, the N1 and P1 waves were 2,147.2 ± 420.4 and 1,266.9 ± 531.6, respectively.

Topical application of EHNA. We then evaluated the effects of adenosine receptor blockade (A₁ and A₂) in an experimental paradigm, in which adenosine concentrations were increased by topically applied EHNA, an adenosine deaminase inhibitor, during SNS. Adenosine deaminase degrades adenosine to vasoinactive inosine (36) and therefore with neuronal activation, blockade of adenosine deaminase would result in increased levels of adenosine in the cerebral interstitium (27). In artificial CSF, stimulation of the contralateral sciatic nerve caused a 27.6 ± 3.2% (n = 10) increase in pial arteriolar diameter. Topical superfusion of EHNA (1 and 10 μmol/l) for 30 min did not significantly affect resting arteriolar diameter, MABP, Pa CO₂, or Pa O₂. However, after the 30-min exposure period to EHNA, the pial arteriolar response to SNS was significantly potentiated (P < 0.05; n = 10, Fig. 5A). The effect of EHNA was irreversible, even after prolonged washout of the subwindow space with artificial CSF. Presumably, EHNA is causing a larger increase in extracellu-
lar concentrations of adenosine during SNS. To test this hypothesis, we studied the effect of Theo on the potentiating effects of EHNA. We found that the EHNA-induced potentiation of the SNS response was significantly ($P < 0.05$; $n = 5$; Fig. 5B) attenuated by systemic administration of Theo (0.05 μmol/g ip).

Topical application of EHNA had no effect on CO$_2$ reactivity of pial arterioles. In artificial CSF, before treatment with EHNA, CO$_2$ reactivity was 1.65 ± 0.41%/mmHg. After topical application, CO$_2$ reactivity was not significantly different at 1.60 ± 0.38%/mmHg. Topical application of DPCPX also had no significant effect on SSEPs. In artificial CSF, before DPCPX application, the amplitude of the N$_1$ and P$_1$ waves were $-1,211.3 ± 445.2$ and $1,201.2 ± 120.7$, respectively. After topical application of DPCPX, the N$_1$ and P$_1$ waves were $-1,131.7 ± 392.9$ and $993.8 ± 287.0$, respectively.

**DISCUSSION**

In the present study, we analyzed the response of the cerebrovascular microcirculation to adenosine receptor blockade during neuronal activation induced by con-

(0.01, 0.10, and 1 μmol/l) had no effect on resting arteriolar diameter, MABP, P$_{CO_2}$, or P$_{O_2}$ (Table 1).

During SNS, vasodilatation was potentiated in the presence of DPCPX (Fig. 6). Thirty minutes of topical superfusion of the lowest concentration of DPCPX (0.01 μmol/l) produced a slight but statistically insignificant potentiation of the SNS-induced vasodilatation (control, 26.1 ± 6.9%; DPCPX, 32.6 ± 12.2%; $n = 5$). However, topical application of higher concentrations of DPCPX (0.10 and 1 μmol/l) produced significant potentiation of the vascular response to SNS (Fig. 6; $P < 0.01$). For example, topical application of 0.1 μmol/l DPCPX caused a 73% potentiation of the SNS-induced vasodilatation ($P < 0.01$; $n = 5$). With 1 μmol/l DPCPX, the observed potentiation of the vascular response to SNS was persistent and irreversible, even after 60 min of washout with artificial CSF.

Topical application of DPCPX had no effect on hypercarbic vasodilatation. Under control conditions, CO$_2$ reactivity was 1.65 ± 0.41%/mmHg. After topical application, CO$_2$ reactivity was not significantly different at 1.60 ± 0.38%/mmHg. Topical application of DPCPX also had no significant effect on SSEPs. In artificial CSF, before DPCPX application, the amplitude of the N$_1$ and P$_1$ waves were $-1,211.3 ± 445.2$ and $1,201.2 ± 120.7$, respectively. After topical application of DPCPX, the N$_1$ and P$_1$ waves were $-1,131.7 ± 392.9$ and $993.8 ± 287.0$, respectively.

![Fig. 5. A: effect of topically applied erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) on pial arteriolar dilation during sciatic nerve stimulation, $n = 10$. B: effect of systemically administered Theo (0.05 μmol/g ip) on EHNA-induced potentiation of the pial response to sciatic nerve stimulation, $n = 5$; All values are means ± SD. *$P < 0.05$ vs. Control; **$P < 0.01$ vs. EHNA.](http://ajpheart.physiology.org/)

![Fig. 6. Effect of topically applied 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on pial arteriolar dilation during sciatic nerve stimulation. All values are means ± SD, $n ≥ 5$; *$P < 0.01$ vs. Control.](http://ajpheart.physiology.org/)
tralateral SNS and report the effects of both A₁ and A₂ adenosine receptor antagonists. Topical application of 8SPT, a lipophobic A₁ and A₂ antagonist, significantly attenuates pial vasodilatation during SNS, whereas systemic administration had no effect. In contrast, systemic administration of Theo, which penetrates the blood-brain barrier (BBB), nearly abolished the SNS-induced vasodilatation. Systemically administered ZM-241385, a relatively selective A₂A antagonist, also significantly attenuated vasodilatation during SNS, whereas topical application had no effect. Topical application of the highly selective A₁ receptor antagonist DPCPX markedly potentiated the pial arteriolar response to SNS. We also tested the effect of EHNA, an adenosine deaminase inhibitor, on the SNS response. EHNA significantly potentiated vasodilatation during SNS. In addition, the EHNA-induced potentiation of the vascular response was significantly blocked by Theo.

Adenosine has been proposed as a regulator of CBF during increased cortical activity (8, 22, 23). In the study by Ko et al. (22), topical application of Theo (5 μmol/l) caused a significant reduction in arteriolar response to SNS. These investigators also reported that superfusion of adenosine uptake inhibitors dipyridamole and inosine caused a significant potentiation of the vascular response to SNS. A more recent study by Dirmagl et al. (8) reported that topical Theo caused a 41% reduction of laser-Doppler flow during cortical activation by whisker stimulation. This magnitude of reduction is similar to the pial arteriolar measurements by Ko et al. (22) and is quite comparable to the reduction (43%) in the arteriolar response to SNS by topical 8SPT reported in the present study. In addition, we noted that systemically administered 8SPT had no effect on SNS-induced vasodilatation. However, 8SPT is a polar molecule and was not expected to penetrate the BBB (4). Our pharmacokinetics studies confirmed that after intraperitoneal administration, CSF levels of 8SPT were negligible (~0.02 μmol/l), whereas plasma concentrations were significantly elevated. The lack of effect of systemic 8SPT on SNS-induced vasodilatation parallels observations on adenosine-induced vasodilatation of intraparenchymal arterioles in vitro (37) and confirms that cerebral endothelial adenosine receptors are not important in mediating increases in CBF during neuronal activation. This is in contrast to the coronary circulation where endothelial adenosine receptors have been implicated in increases in coronary blood flow (17, 30).

The vascular response to SNS was almost entirely abolished by systemic administration of Theo, a novel observation. Theo was chosen for several reasons. First, compared with caffeine, affinity for the A₂ receptor is greater than A₁ (20). Second, the pharmacokinetics of Theo are well characterized (29). For example, we (29) have shown that intraperitoneal administration of Theo (0.05 μmol/g), leads to micromolar concentrations in the CSF but well below concentrations expected to affect phosphodiesterase inhibition, catecholamine, and calcium activation. We would surmise that the greater suppression of the arteriolar response to SNS, with intraperitoneal versus topical application of Theo, may be related to better distribution of the antagonist in the cerebral interstitium after intraperitoneal injection. Neither Theo nor 8SPT significantly affected SSEPs. Thus the effects of these adenosine antagonists during SNS are related to a primary action on the vasculature and not an indirect neurophysiological depression of the evoked response.

In contrast with the present and previous studies (8, 22, 23), Northington et al. (38) were unable to alter the CBF response to SNS using 8SPT. They utilized a modified microdialysis cannula equipped with a hydrogen clearance electrode to deliver 8SPT. The inability of Northington et al. (38) to affect the CBF response to SNS can be attributed to several factors. First, these investigators performed their studies on CBF shortly (60 min) after penetrating the brain with a microdialysis cannula, which has clearly been shown to alter the BBB (46). Thus 8SPT introduced into the brain tissue with a surrounding compromised BBB would be exposed to the large sink of the cerebral circulation, significantly decreasing its concentration and thereby attenuating or negating its actions. Second, the spatial resolution of the hydrogen clearance electrode and the distribution of drugs delivered through the microdialysis probe may not be concordant, especially in a situation where the BBB is altered and differentially affecting hydrogen clearance and drug distribution. Third, Northington et al. (38) utilized laser Doppler methods to measure CBF over a 30-s period of SNS. They noted a pattern of continuously rising blood flow, which is different from the pial arteriolar response noted by our laboratory (32, 33) and others (2). Northington et al. (38) attached physiological significance to their observations but other explanations are possible. Ngai et al. (33) found that the laser Doppler flow pattern is identical to changes in pial arteriolar dilation during SNS. A response pattern of an initial peak, followed by a decline to a steady, smaller response plateau was consistently evoked with stimulus parameters of 0.2–0.3 V, 5 Hz, and 0.5-ms pulses (32). The response became irregular and inconsistent if stronger stimuli were used (e.g., by increasing pulse duration), partly because of the accompanying fluctuations in blood pressure, as we previously described (32). Northington et al. (38) did not specify their stimulus parameters. Ngai et al. (33) showed that increasing pulse duration from 0.5 to 5 ms evoked a steadily rising response with no initial peak. This response pattern is similar to that reported by Northington et al. (38).

One argument raised by Northington et al. (38) against the role of adenosine in the regulation of CBF during increased cortical activity is that there is no oxygen deficit during SNS and therefore no signal for adenosine release. Previous studies (3, 7) in cardiac tissue have shown that adenosine production is linked to tissue PO₂. This relationship between PO₂ and adenosine production was the basis of Berne’s adenosine hypothesis (5). The argument raised by Northington et al. (38) is based on PET scan studies in brain, which...
were unable to demonstrate hypoxia during neuronal activation (11). However, recent optical imaging studies (25) demonstrate that during cortical activation, there is indeed a transient dip in oxygenation and an increase in deoxyhemoglobin. The optical imaging technique has a much faster temporal resolution compared with PET scanning and may explain the failure of PET measurements to detect this transient hypoxic period.

The present study is the first to demonstrate potentiation of the vascular response to cortical activation with A1 receptor blockade. The A1 subtype is found in high density at synaptic terminals throughout the brain (20). Presynaptic receptors presumably act to inhibit transmitter release (13), whereas postsynaptic receptors inhibit neuronal firing (44). Thus, activation of the A1 receptor subtype leads to a reduction of CNS activity, whereas blockade of this receptor subtype would result in disinhibition, i.e., activation (1). Because DPCPX is a highly selective antagonist for the A1 receptor subtype (45), our data suggests that the potentiated CBF response during SNS is due to a reduction of the neuroinhibitory actions of endogenously released adenosine on A1 receptors.

Our data that A1 blockade results in hyperemia with SNS may also explain the recent observation by Gotoh et al. (14), who studied the influence of caffeine on CBF during brain activation by whisker-barrel stimulation. These investigators found that in contrast to the trigeminal nuclei in the brain stem where systemically administered caffeine attenuated increases in CBF during whisker-barrel stimulation, there was only a downward trend in CBF in the thalamic relay nucleus, and surprisingly, a suggestion of hyperemia in the barrel field cortex. These findings in barrel field cortex are in variance to the observations in the present study, which noted an attenuation in CBF with neural activation in the presence of Theo. The A2A/A1 affinity ratio for caffeine is 1.7, in contrast with a ratio of 3.0 for Theo. Thus, caffeine would be more likely to cause neuronal activation (by disinhibition) than Theo. Indeed, Gotoh et al. (14) did in fact find that, “...in the barrel cortex, caffeine statistically significantly (P < 0.05) enhanced the percentage of increase in ICMBf to stimulation (Fig. 3).” Therefore, we believe that the failure of Gotoh et al. (14) to attenuate the CBF in barrel field cortex during whisker barrel stimulation is related to their use of caffeine rather than Theo and the resultant greater blockade of A1 receptors compared with A2 receptors.

We also evaluated the effect of ZM-241385, a relatively selective antagonist for the A2A receptor subtype. Similar to Theo, topical application of ZM-241385 was less effective than systemic application. The dose range of ZM-241385 applied systemically (0.1–1.0 mg/kg) in this study was based on reports (43) on the pharmacodynamics of ZM-241385 after enteric administration. Poucher et al. (43) reported that intravenous administration of ZM-241385 (10 mg/kg) resulted in plasma concentration <1 μmol/l at 60-min postadministration. Thus, in our experiments, the plasma concentration of ZM-241385 after 0.1, 1, and 5 mg/kg is likely to be <10, 100, and 500 nmol/l, respectively. However, the cerebral interstitial concentrations of ZM-241385 after these doses is not known, and is probably much less than would be predicted by the plasma levels reported in the study by Poucher et al. (43). The affinity of ZM-241385 for the various adenosine receptor sub-types is well documented (40, 42). ZM-241385 has an ~10-fold greater affinity for the A2A versus the A2B subtype and the reported inhibitor constant (Ki) for ZM-241385 for A2A and A2B are 0.8 nM and 50 nmol/l, respectively (40, 43). Moreover, ZM-241385 is 300- and 10,000-fold more active at the A2A than at the A1 and A3 receptor, respectively. In the present study, intravenous administration of ZM-241385 at a low dose (0.1 mg/kg) had no effect on vasodilatation during SNS. After intravenous doses of 1 or 5 mg/kg, ZM-241385 significantly attenuated the vascular response to SNS by 54% and 79%, respectively. We would surmise that at the lowest dose (0.1 mg/kg), ZM-241385 is not accumulating in the cerebral interstitium in high enough amounts to affect the A2A receptors. At a dose of 1 mg/kg, the effect of ZM-241385 is probably predominantly A2A with perhaps some influence on the A2B subtype. However, at the highest dose (5 mg/kg) ZM-241385 is probably affecting both the A2A and A2B subtypes. Furthermore, as we have recently demonstrated (31) in an in vitro microvascular preparation, ZM-241385 is not capable of a complete blockade of high (>10 μmol/l) concentrations of adenosine. We would conclude, however, that ZM-241385 is affecting vasodilatation during neuronal activation through blockade of vascular A2 receptors, presumably the A2A subtype on vascular smooth muscle.

Topical application of EHNA, an adenosine deaminase inhibitor, significantly potentiated the SNS-induced pial vasodilation. This novel observation of the potentiating effect of EHNA on the SNS response is consistent with an increase in endogenous adenosine concentrations during neuronal activation and greatly strengthens the contention that adenosine is involved in the vasodilatation during neuronal activation. In contrast, EHNA had no effect on resting pial vessel diameter. EHNA has also been reported (41) to inhibit cGMP-phosphodiesterase in the myocardium, an effect that may lead to a nonreceptor-mediated potentiation of vasodilatation during SNS. In the present study, this appears to be unlikely given that adenosine receptor blockade with Theo, at a concentration that would not affect phosphodiesterase, completely reversed the potentiation by EHNA.

Adenosine thus appears to be involved in CBF regulation during neuronal activation. Our present observation of almost total elimination of the vascular response during SNS by systemic Theo and ZM-241385, suggests that adenosine may be the dominant factor involved in CBF increases during neuronal activity in the brain, although other factors and mechanisms such as nitric oxide (NO) may also play a role. However, studies (39) proposing to demonstrate a regulatory role for NO fail to consider a primary neurophysiological...
effect of NO synthase blockade on neuronal activity. For example, Ngai et al. (34) noted significant depression of SSEPs (both N and P waves) with N\textsuperscript{G}-nitro-L-arginine (L-NNA), which precede suppression of the vascular response to SNS. Furthermore, this SSEP suppression could be reversed by application of arginine, which is the substrate for NO production. The report by Ngai et al. (34) has subsequently been challenged in a study by Lindauer et al. (24) where topical application of L-NNA had no effect on SSEPs during vibrissal or forepaw cutaneous stimulation. However, a followup study by Ngai et al. (35) reconfirmed significant attenuation of SSEPs with topical L-NNA during SNS or hind paw stimulation. Moreover, the recent study by Ngai et al. (35) outlines significant methodological differences between these studies and demonstrated that the lack of effect reported by Lindauer et al. (24) can be explained by the positioning of their recording electrode. Thus the inhibitory effects of NO synthase inhibitors on CBF increases during neuronal activity may be secondary to a primary effect on neuronal transmission or even to an effect on pial vessels, as noted by Ngai et al. (34). In contrast, the present study demonstrates that the attenuated response of the cerebral circulation during SNS caused by 8SPT, Theo, or ZM-241385 is a primary effect on the cerebral blood vessels and not a secondary effect related to alteration in neuronal activity (i.e., SSEPs). The lack of effect on glucose metabolism by caffeine, has also been recently demonstrated by Gotoh et al. (14).

In summary, we have shown that extraluminal adenosine receptor blockade significantly attenuates the vascular response to SNS, whereas intraluminal blockade with systemic 8SPT was ineffective. Moreover, the selective A\textsubscript{2A} antagonist ZM-241385 significantly attenuated the SNS response suggesting the involvement of the A\textsubscript{2A} subtype. However, the A\textsubscript{2B} receptor subtype may also be involved. Furthermore, selective A\textsubscript{1} receptor blockade leads to a secondary potentiation of the vascular response to SNS, probably due to primary disinhibition of neuronal activity. Thus the results of present study provide further evidence for a role of adenosine in the regulation of CBF during increased cortical activity.

This study was supported by National Institutes of Health Grant NS-21076.

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