The Role of Adenosine in CBF Increases During Hypoxia in Young vs Aged Rats

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SUMMARY The role of adenosine in the regional cerebral blood flow (rCBF) response to hypoxia was evaluated in young (6 month) and aged (26–28 month) F344 rats using theophylline, an adenosine antagonist. Regional CBF was measured with radioactive microspheres under control anesthetized conditions (70% N₂O, 30% O₂) and at two levels of hypoxia (CaO₂ = 8.7–9.0 ml/100 ml-1 and 3.2–3.7 ml/100 ml-1). Without theophylline infusion, CBF increases were similar between young and aged rats during moderate hypoxia but were increased more in young during severe hypoxia. Intracerebrovascular theophylline infusion significantly attenuated the increase in CBF during both moderate and severe hypoxia and decreased the difference between young and aged rats. Theophylline infusion produced no significant effect on the increase in CBF produced by hypercapnia, indicating the specificity of the treatment for hypoxic induced CBF changes and adenosine release. Intracerebrovascular infusion of adenosine had no effect on CBF, presumably due to the presence of the blood brain barrier. The results suggest that adenosine plays a major role in CBF increases during both moderate and severe hypoxia and in the difference in response to hypoxia between young and aged rats.

Stroke Vol 15. No 1, 1984

ALTHOUGH CEREBRAL BLOOD FLOW (CBF) remains relatively constant under several experimental conditions, it may increase several fold during hypoxia.1,2 Recently, we have compared the cerebrovascular response of young and aged rats to hypoxic challenges and have observed an attenuated increase in CBF in aged animals as the degree of hypoxia becomes more severe.3 The mechanism or mechanisms by which CBF increases during hypoxia is unclear. Although neural mechanisms initiated by stimulation of peripheral chemoreceptors have been suggested to produce increases in CBF,4 Kontos et al5 have indicated cerebrovascular resistance changes during hypoxia are controlled locally within the brain tissue. The metabolic response to changes in arterial oxygen content and release of locally active vasoactive substances produced within brain tissue may also mediate increases in CBF produced by hypoxia. Adenosine, H⁺, K⁺ and lactate have all been suggested to provide this chemical link between cerebrovascular resistance and tissue oxygen supply.6-10 The role of tissue pH changes in mediating hypoxic induced short term CBF increases has been questioned,11 however, and in recent studies, we found no evidence to indicate a major role for brain tissue pH changes influencing CBF increases during brief hypoxic challenges or for differences in cerebrovascular responses between young and aged rats.3 Adenosine appears to be the only factor which experimentally has fulfilled all the criteria for mediating CBF changes during hypoxia. Tissue adenosine concentration changes correlate with increases in CBF during ischemia or hypoxia.12,13 Adenosine will dilate cerebral vessels when applied directly to pial arteries14 and intracerebrovascular infusion of theophylline, an adenosine antagonist, attenuates the increase in CBF induced by hypoxia in dogs.15 The current experiments were carried out to analyze the role of adenosine in the cerebrovascular response to hypoxia in rats and in the difference in response between young and aged animals.

Methods

Male F-344 rats, 6 months and 26–28 months old, were used in these experiments (Charles Rivers Inc.). For surgery rats were anesthetized with halothane, tracheotomized and artificially ventilated with 1% halothane in 70% N₂O and 30% oxygen using a Harvard small animal respirator. PE50 tubing catheters filled with heparinized saline were inserted into both femoral arteries and a femoral vein. A catheter was also inserted into the left ventricle via the right carotid artery. Pressure pulses were monitored using a Hewlett Packard pressure transducer and strip chart recorder to insure proper placement of the ventricular catheter. The left carotid artery was exposed and a catheter inserted retrograde into the external carotid to end at the origin of the internal carotid artery. This catheter was used for intracerebrovascular theophylline infusion. Following the completion of all surgical procedures, halothane was removed from the inspired gases and the rat allowed 30 minutes to stabilize. Arterial PCO₂ was adjusted to 35–40 mmHg. Rectal temperature was measured with a Yellow Springs Inc. thermistor probe and maintained at 37°C using overhead heat lamps. Mean blood pressure was recorded continuously from a femoral artery catheter.

Microspheres

Fifteen micron microspheres, labelled with cobalt-57, ruthenium-103 or scandium-146 (New England Nuclear) were used in these studies. Stock solutions containing 500,000 microspheres/ml-1 were suspend-
ed in isotonic saline with 0.01% Tween-80. Ventricular pressure pulses were monitored before each microsphere injection. Microspheres were vortexed for one minute, 0.2 ml withdrawn (100,000 microspheres), injected into the left ventricle via the ventricular catheter (dead space = 0.06 ml) and flushed in with 0.2 ml saline over 20 seconds. Starting immediately before each microsphere test and continuing 45 seconds after the end of each injection, blood was withdrawn from a femoral artery at a rate of 0.4 ml·min⁻¹ using a Harvard infusion-withdrawal pump. Arterial blood samples were taken at the end of each test for measurement of blood gases and pH, using an IL 1303 blood gas analyzer and oxygen content was measured using an IL282 co-oximeter. Oxygen content was calculated as the sum of the co-oximeter value plus 0.03 times the PO₂ in each blood sample. Mean arterial blood pressure was measured continuously throughout the microsphere test from the second femoral artery to ensure blood pressure did not change appreciably.

At the end of the last microsphere test the rat was sacrificed, the brain removed and sectioned into left and right cortical and subcortical samples and weighed. The activity of each microsphere in brain and blood samples was analyzed using a Nuclear Chicago 1035 gamma counter and a Nuclear Data 600 multichannel analyzer. CBF was analyzed according to the methods of Heymann et al.¹⁶

Determining the infusion rate of theophylline

It is reported that intracerebrovascular infusion of theophylline in high doses produces decreases in cerebrovascular resistance.¹⁵ Preliminary experiments were carried out here to determine an intracerebrovascular infusion rate of theophylline which may inhibit the cerebrovascular effects of adenosine but which would not alter control CBF values. CBF was measured under three conditions in these experiments. The first under control anesthetized conditions with no infusion of theophylline. The second during the intracerebrovascular infusion of 1 µg·min⁻¹ theophylline and the third during the infusion of 5 µg·min⁻¹. Theophylline was dissolved in saline for intracerebrovascular infusion and the volume infusion rate was maintained at 0.1 ml·min⁻¹ for both 1 and 5 µg·min⁻¹ theophylline infusions. CBF was measured with radioactive microspheres for each test. Theophylline infusion was carried out for ten minutes before each microsphere injection. It was observed that an infusion of 5 µg·min⁻¹ but not 1 µg·min⁻¹ produced an increase in CBF. Therefore the lower infusion rate (1 µg·min⁻¹) was used in these experiments.

Induction of Hypoxia

A modification of Levine's model of hypoxic-ischemia,¹⁹ consisting of unilateral carotid artery occlusion was used in these experiments. Hypoxia was induced by substituting oxygen with nitrogen in the inspired gases. Arterial oxygen content (CaO₂) was decreased to approximate levels of 9 and 3.5 ml O₂·dl⁻¹ in control and theophylline treated young and aged rats. CO₂ was added to the inspired gases during hypoxia to maintain PaCO₂ at approximately 35 mmHg. Hypoxia was maintained for 5–7 minutes before each microsphere test. Theophylline was infused intracerebrovascularily at a rate of 1 µg·min⁻¹ in a volume of 0.1 ml·min⁻¹ starting 5 minutes before the induction of hypoxia and continuing until the end of the microsphere test. Microsphere tests were performed randomly in young and aged rats under control and graded hypoxic conditions. Arterial blood pressure was maintained above 100 mmHg in all tests. At the end of each microsphere test arterial samples were drawn for measurement of blood gases, pH and oxygen content.

The effect of theophylline infusion was also tested during the induction of hypercapnia. In these tests, young F344 rats were prepared as described above. Arterial PCO₂ was increased by adding CO₂ to the inspired gases. CBF was measured in these rats under control anesthetized conditions and 7 minutes after the addition of CO₂ to the inspired gases. One group of rats were infused with theophylline at a rate of 1 µg·min⁻¹, starting 5 minutes before the induction of hypercapnia. A second group of rats received the same treatment but were infused with saline at a rate of 0.1 ml·min⁻¹ instead of theophylline.

Intracerebrovascular infusion of adenosine

Adenosine was infused intracerebrovascularily in order to determine possible dose-dependent differences in CBF responsiveness between young and aged rats. Preliminary experiments were carried out in young F344 rats. Rats were prepared for intracerebrovascular infusion as described above. CBF was measured in these rats using radioactive microspheres under each of three test conditions: 1). Control anesthesia; 2). Intracerebrovascular infusion of 1 µg·min⁻¹ adenosine; 3). Intracerebrovascular adenosine infusion at 10 µg·min⁻¹. Adenosine was infused for ten minutes before each microsphere injection. Adenosine was dissolved in Kreb’s solution and the pH adjusted to 7.4 with sodium bicarbonate for intracerebrovascular infusion. The volume rate of the infusion was maintained at 0.1 ml·min⁻¹ for each test. Neither of the adenosine infusions produced a significant change in CBF in these rats. Therefore, these experiments were not repeated in aged rats.

Data are reported as mean ± SE and statistics performed included unpaired t-tests and a linear model analysis of variance.

Results

Initial studies were carried out to determine a dose of theophylline which, when infused intracerebrovascularily, may provide adenosine blocking capability but would have no significant effect on CBF. In four rats tested under control anesthetized conditions, total CBF averaged 72 ± 7 ml·100g⁻¹·min⁻¹. An intracerebrovascular infusion of theophylline in a dose of 1 µg·min⁻¹ produced CBF values of 77 ± 5 ml·100g⁻¹·min⁻¹ and an infusion of theophylline at a rate of 5 µg·min⁻¹ produced CBF values of 140 ± 10 ml·100g⁻¹·min⁻¹.
An infusion rate of theophylline of 1 μg·min⁻¹ was chosen for these studies. The effect of theophylline infusion on CBF changes during hypoxia are shown in figure 1. Arterial blood pressure and blood gases for these studies are shown in table 1. Similar levels of hypoxia were induced in young and aged rats, compared in terms of arterial oxygen content, although arterial PO₂ changes were different at the mid hypoxic level (CaO₂ = 8.8–9.5 ml·100 ml⁻¹). Arterial blood pressure was maintained above 100 mmHg for all tests in both test groups; arterial PCO₂ was maintained at approximately 38 mmHg during hypoxia by the addition of CO₂ to the inspired gases and arterial pH decreased to similar levels in young and aged rats at each hypoxic level. CBF increased in both young and aged rats during hypoxia. These changes were greater in young than in aged rats at the most hypoxic level (p < .05). CBF increases were greater in the left hemisphere during hypoxia compared to the right (carotid ligated) side. Theophylline infusion attenuated the increases in CBF during hypoxia in all cerebral tissues measured in both young and aged rats and abolished the difference in flow changes between young and aged at the most hypoxic level. CBF changes during hypercapnia and theophylline infusion are shown in figure 2 with blood gas changes described in table 1. Hypercapnia increased CBF and these increases were not significantly changed by theophylline infusion (p > .05). The effect of intracerebrovascular infusion of adenosine on CBF is shown in table 2. Adenosine infusion in doses of 1 and 10 μg·min⁻¹ produced no significant change in CBF (p > .10).

Discussion

Results presented here indicate an attenuated cerebrovascular response in aged rats to severe but not moderate hypoxic challenges, in agreement with a previous report. Cerebrovascular adenosine receptor inhibition with theophylline infusion significantly attenuated the CBF increase to moderate and severe hypoxia and decreased the difference in cerebrovascular responsiveness between young and aged rats to severe hypoxic challenges. These data are consistent with Emerson and Raymond, who reported an intracerebrovascular infusion of theophylline completely inhibited the cerebrovascular response to moderate hypoxia (PaO₂ = 47 mmHg) and partially reversed the CBF response to severe hypoxia (PaO₂ = 20 mmHg). In that study, the failure of theophylline to completely reverse the effects of severe hypoxia were attributed to at least three possibilities. First, that theophylline did not reach all vascular compartments. Second, that the concentration of theophylline was not adequate to inhibit the amounts of adenosine released during severe hypoxia, and third, that other factors such as H⁺, K⁺ and/or O₂ may contribute to the cerebrovascular response to a greater degree as hypoxia becomes more profound. The first possibility is not likely since theophylline penetrates the blood brain barrier and is readily accessible to the vascular compartments. It is possi-

![Figure 1. CBF changes during hypoxia in control (PRE) and theophylline (POST) treated rats. Blood bases and n for each group shown in table 1. Significant difference between control treated young and aged rats was observed in each tissue measured at the most hypoxic level. (CaO₂ = 3.2–3.3 ml·100 ml⁻¹. p < .05).](image-url)
ble, however, that the dose of theophylline used was not adequate to completely inhibit the effects of adenosine as hypoxia became more severe. The infusion rate of theophylline used was one which had minimal cerebrovascular effects alone. Above this dose, theophylline produced vasodilation and increased CBF. This is consistent with other reports of the effects of theophylline in brain and heart tissue and suggests that an infusion rate of the drug which is high enough to block adenosine released during hypoxia cannot be achieved because of the direct vascular effects of theophylline. Other factors may be involved in the CBF response to hypoxia, particularly at extreme hypoxic levels. There is a correlation between brain tissue pH changes in CBF during hypoxia and local pH changes have been shown to produce dilatation of pial arteries. More recent data indicate that brain tissue pH changes are not the major factor mediating the CBF response to hypoxia, although some role cannot be ruled out. Potassium ion is another candidate for mediating CBF changes during hypoxia. Increases in brain tissue K⁺ have been measured in CSF during severe but not moderate hypoxia and similar concentrations of the ion as produced by severe hypoxia, applied topically to pial arteries, produces vasodilation.

Measurement of tissue adenosine concentration

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Arterial Blood Gases and pH During Theophylline Infusion, Hypoxia and Hypercapnia</th>
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</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>Arterial Blood Pressure (mm Hg)</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
</tr>
<tr>
<td>Young</td>
<td>10</td>
</tr>
<tr>
<td>Aged</td>
<td>9</td>
</tr>
<tr>
<td>Theophylline treated</td>
<td>7</td>
</tr>
<tr>
<td>Young</td>
<td>10</td>
</tr>
<tr>
<td>Aged</td>
<td>12</td>
</tr>
<tr>
<td>Hypercapnia (young rats only)</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
</tr>
<tr>
<td>Theophylline</td>
<td>8</td>
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<tr>
<td>Aged</td>
<td>8</td>
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<tr>
<td>Hypercapnia (young rats only)</td>
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<td>Control</td>
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<tr>
<td>Theophylline</td>
<td>12</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Arterial Blood Pressure, Blood Gases and Cerebral Blood Flow Changes During Intracerebrovascular Adenosine Infusion (n = 8)</th>
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</thead>
<tbody>
<tr>
<td>Intracerebrovascular Adenosine</td>
<td>Control</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>144±5</td>
</tr>
<tr>
<td>Arterial PO₂ (mm Hg)</td>
<td>109±6</td>
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<tr>
<td>Arterial PCO₂ (mm Hg)</td>
<td>37.6±1.4</td>
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<tr>
<td>Arterial pH</td>
<td>7.37±0.02</td>
</tr>
<tr>
<td>Cerebral blood flow (ml·100 g⁻¹·min⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Right cortex</td>
<td>146±11</td>
</tr>
<tr>
<td>Left cortex</td>
<td>178±14</td>
</tr>
<tr>
<td>Right subcortex</td>
<td>94±9</td>
</tr>
<tr>
<td>Left subcortex</td>
<td>118±12</td>
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</table>
changes during hypoxia as reported by Winn et al. support the hypothesis that adenosine plays a role in the regulation of CBF during cerebral hypoxia. Brain tissue adenosine concentration increases significantly even after 30 seconds of hypoxia ($\text{PaO}_2 = 14 \text{ mmHg}$). This is consistent with previous reports from that laboratory, and with other reports of increased brain tissue adenosine during challenges such as ischemia or hypotension. On the other hand, Rehncrona et al. have reported no significant change in brain tissue adenosine concentration during hypoxia. However, the in situ funnel freezing method used in that study has been criticized because it may not freeze brain tissue fast enough to provide an accurate measure of actual tissue adenosine levels. More recent reports indicate that brain adenosine concentration increases during both moderate and severe hypoxia and these changes correlate with changes in cerebrovascular resistance. It is possible that an altered response to adenosine during hypoxia represents a specific change in responsiveness of the aged cerebrovasculature to the molecule or a generalized decrease in reactivity of cerebral blood vessels to vasoactive substances in general. Other reports indicate a decreased responsiveness of aged cerebrovasculature to both hypercapnic and hypocapnic challenges. This may indicate a generalized decrease in cerebrovascular responsiveness of aged arterial vessels to metabolic stimuli. Our results would suggest that cerebrovascular reactivity, but not sensitivity to hypoxic challenges may be decreased as a function of aging, since CBF increases are similar between young and aged at moderate levels of hypoxia and are attenuated in aged only as hypoxia becomes more severe. While an altered cerebrovascular-adenosine response is suggested between young and aged rats, we cannot at the present time determine whether differences in the CBF response to hypoxia at severe levels of hypoxia represent a difference in cerebrovascular reactivity to adenosine stimulation or a difference in adenosine production at severe hypoxic levels. An attempt was made to answer this question comparing CBF responses to intracerebrovascular infusion of adenosine. A moderate increase in CBF was observed in the left side of the brain with no change or possibly a decrease in CBF in the right cerebral hemisphere during adenosine infusion. These effects may have represented some cerebrovasodilation in the left hemisphere and a possible steal of CBF from the right (carotid ligated) hemisphere. However, these changes did not reach a level to be considered statistically significant. It is also possible that adenosine infusion increased CBF but that the effect was transient and not easily detectable by the microsphere methods used in these experiments, as suggested by the results of Tagashira et al. Statistically, however, the data support previous reports that intracarotid infusion of even high doses of adenosine produce no change in CBF, presumably due to the presence of the blood brain barrier. The question of altered brain adenosine production vs altered cerebrovascular reactivity in young vs aged subjects during hypoxia may be answered in the future by measurement of brain tissue adenosine concentration changes in young and aged rats during graded levels of hypoxia or by altering cerebrovascular permeability to allow access of adenosine to receptors within cerebrovascular tissue.

References

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Cerebrovascular Response to Hypoxia in Young vs Aged Rats

WILLIAM E. HOFFMAN, PH.D., RONALD F. ALBRECHT, M.D., AND DAVID J. MILETICH, PH.D.

SUMMARY Cerebrovascular responses of young and aged rats were tested to graded levels of hypoxia using a modification of the Levine ischemic-hypoxic rat model in which one carotid artery was ligated. Rats were anesthetized with 70% N₂O, 30% O₂ and cortical and subcortical cerebral blood flow (CBF) were measured with radioactive microspheres. CBF and cerebral cortical oxygen consumption (CMRO₂) were measured under control conditions and during hypoxia with arterial oxygen content maintained at approximately 9, 5 and 3 ml dl⁻¹. CBF responses in cortical and subcortical tissues were similar between young and aged under control conditions and during moderate hypoxia (CaO₂ = 9 ml dl⁻¹). Maximum cerebrovascular responses to severe hypoxia were greater in young than in aged rats and these trends were significant in both ligated and uninjured cortical tissue (p < 0.05). CMRO₂ was maintained at control levels during moderate hypoxia but decreased significantly more in aged than in young rats when CaO₂ was decreased to 3 ml dl⁻¹. These results suggest that baseline CBF and the sensitivity of cerebrovascular receptors to moderate hypoxia are similar in young vs aged rats but that maximum reactivity to severe hypoxia is attenuated in aged subjects. CBF measured after one minute of hypoxia, before the induction of brain tissue acidosis, produced no significant change in the CBF response to hypoxia or in the difference between young and aged rats. Brain tissue pH changes do not appear to be the major factor for mediating CBF increases during hypoxia in young or aged rats, although it may interact with other mediators of the response.

ANESTHETIC MANAGEMENT of the aged patient is often complicated by decreased cardiovascular reserve and preoperative cardiorespiratory disease.¹⁻² This increases the risk of hypoxic-ischemic episodes during anesthetic procedures and produces an increased incidence of postoperative hypoxemia with advancing age.²⁻³ It is important to know whether changes occur in the aged cerebrovasculature may alter the sensitivity or reactivity of these vessels to hypoxic or metabolic stimuli and further increase the risk of hypoxic-ischemic brain damage. However, little is known about these possible changes. Several reports suggest that the cerebral blood flow (CBF) response to hypercapnic and hypoxic stimuli are attenuated in aged compared to young subjects.⁴⁻⁵ On the other hand, Haining et al.⁶ reported no difference in the initial CBF response to moderate hypoxia (PaO₂ = 60–70 mm Hg) in young vs aged rats. These results suggest there may be differences in sensitivity and/or reactivity to various cerebral metabolic stimuli in young vs aged subjects. In these experiments we have tested cerebrovascular changes to graded levels of arterial hypoxia and evaluated the possibility that tissue pH changes may mediate these responses. The results indicate that cerebrovascular sensitivity and CBF responses to moderate hypoxic challenges are similar between young and aged rats but reactivity of aged vessels to severe hypoxic challenges is attenuated. Brain tissue pH changes do not play a major role in these responses.

Methods
Male F-344 rats, 6 months and 26–28 months old, were used in these experiments (Charles River Inc.). For surgery rats were anesthetized with halothane, tracheostomized and artificially ventilated with 1% halothane in 70% N₂O and 30% oxygen using a Harvard small animal respirator. PE50 tubing catheters filled with heparinized saline were inserted into both femoral arteries and a femoral vein. A catheter was also inserted into the left ventricle via the right carotid artery. Pressure pulses were monitored using a Hewlett Pack-
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Stroke. 1984;15:124-129
doi: 10.1161/01.STR.15.1.124

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