Adenosine induces initial hypoxic-ischemic depression of synaptic transmission in the rat hippocampus in vivo

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Gervitz, L. M., L. O. Lutherer, D. G. Davies, J. H. Pirch, and J. C. Fowler. Adenosine induces initial hypoxic-ischemic depression of synaptic transmission in the rat hippocampus in vivo. Am J Physiol Regulatory Integrative Comp Physiol 280: R639–R645, 2001.—The present study was designed to investigate the role of adenosine in the hypoxic depression of synaptic transmission in rat hippocampus. An in vivo model of hypoxic synaptic depression was developed in which the common carotid artery was occluded on one side in the urethane-anesthetized rat. Inspired oxygen levels were controlled through a tracheal cannula. Rats were placed in a stereotaxic apparatus for stimulation and recording of bilateral hippocampal field excitatory postsynaptic potentials. The percent inspired oxygen could be reduced to levels that produced a reversible and repeatable depression of evoked synaptic transmission restricted to the hippocampus ipsilateral to the occlusion. Further reduction in the level of inspired oxygen depressed synaptic transmission recorded from both hippocampi. The adenosine nonselective antagonist caffeine and the A1 selective antagonist 8-cyclopentyltheophylline prevented the initial depression in synaptic transmission. We conclude that the initial depression of synaptic transmission observed in the rat hippocampus in vivo is due to endogenous adenosine acting at neuronal adenosine A1 receptors.

hypoxia; ischemia

ONE OF THE EARLIEST RESPONSES to cerebral hypoxic and/or ischemic conditions is a reversible inhibition of evoked synaptic potentials. This early inhibition of evoked potentials is primarily due to a suppression of synaptic function (6, 7, 10, 21, 27). There is substantial evidence from in vitro preparations that the initial reversible loss of synaptic activity during exposure to hypoxia or ischemia-like conditions is due to the release of endogenous adenosine acting at neuronal A1 receptors. Hypoxia and ischemia-like conditions increase the efflux of purines, including adenosine, from the rat hippocampal slice (9, 19). Graded hypoxia results in proportionate changes in both adenosine levels and the depression of the evoked potential (8). Adenosine outflow temporally correlates with the hypoxic depression of the evoked potential (18). Finally, adenosine A1-selective antagonists significantly attenuate the early depression of synaptic transmission in hippocampal slices exposed to either hypoxia or ischemia-like conditions (6, 7, 12).

Although a strong case can be made for adenosine’s role in hypoxic-ischemic cerebral vascular autoregulation (2, 29, 34), current evidence is more equivocal with regard to adenosine’s role in the hypoxic-ischemic inhibition of synaptic transmission in vivo. Presently, there is only indirect evidence suggesting that adenosine modulates hippocampal synaptic transmission during hypoxia-ischemia in vivo. Perhaps the most suggestive evidence of an adenosine-mediated depression of synaptic activity during hypoxia-ischemia in vivo is its ability to inhibit excitatory amino acid release (4, 13, 33). However, the efflux of excitatory amino acids induced by hypoxia-ischemia can originate from both synaptic and nonsynaptic pools (15). Thus it is not clear whether A1-receptor-mediated inhibition of glutamate release reflects an adenosine A1-receptor-mediated inhibition of synaptic transmission or whether this is an indirect effect of adenosine’s other neuroprotective actions.

The purpose of this study was to characterize an in vivo model exhibiting a repeatable and reversible depression of hippocampal synaptic transmission in response to hypoxia. A unilateral carotid occlusion model coupled with systemic hypoxia was found to be appropriate (20). Experiments were designed to examine the role of adenosine in this hypoxic depression of synaptic transmission.

METHODS

Animal preparation. All surgical and experimental procedures followed institutional animal care guidelines. Male Sprague-Dawley rats, weighing 200–300 g, were anesthetized with urethane (1.5 g/kg ip). The right jugular vein was exposed and catheterized with polyethylene (PE-50) tubing for administration of supplemental anesthesia (typically 10% of initial dose). A tracheostomy was performed, and a cannula was inserted to ensure a patent airway. The distal end of the cannula was inserted into a T-tube to allow for the control of the percent inspired oxygen administered. Inspired air was provided on a flow-by basis through the T-tube. During normoxia, the animals were administered 21–25% O2 to maintain normal blood gas values. During hypoxia, the inspired O2 was between 8 and 12% attained by mixing compressed air with N2. The animals were allowed to breathe

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spontaneously, and the flow rate of inspired gas was adjusted to ensure removal of expired air without alteration of ventilation. One common carotid artery was exposed and permanently occluded by ligation with Ethicon 4/0 surgical silk. In some animals, the carotid artery was cannulated proximal to ligature for the purpose of monitoring arterial pressure and obtaining samples for arterial blood gas measurements. Body temperature was monitored by a rectal probe and was maintained between 37 and 38°C using heating pads. Animals were killed while still fully anesthetized by intravenous administration of 2 M KCl.

Electrophysiology. Animals were placed in a stereotaxic apparatus. A midline incision, made just posterior to the eye orbits and extending to the interaural line, was used to expose the skull. Small burr holes (3–4 mm diameter) were drilled in the skull at each of four sites. Concentric bipolar stimulating electrodes were placed 3.0 mm posterior to bregma, 1.6 mm bilaterally to midline, and 2.7 mm below the cortical surface. Recording electrodes, pulled from glass micropipettes and filled with 1 M NaAc, were positioned 5 mm posterior to bregma, 3.2 mm bilaterally to the midline, and 2.5–3.0 mm below the cortical surface. The recording electrodes had a 2–5 MΩ resistance, and 2% Pontamine sky blue was used for position marking.

Evoked field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of CA1. The final position of the recording electrodes was determined by electrophysiological criteria. The criteria consisted of lowering the electrode to record initially a positive-going fEPSP that at times displayed a downward population spike. This waveform is typical of a recording from the stratum pyramidale. The electrode was lowered further until the fEPSP reversed in polarity and reached a maximum amplitude to achieve optimal placement in the CA1 stratum radiatum. Stimulation intensity was then adjusted to ensure that the fEPSP was uncontaminated by the population spike. The fEPSPs were elicited at 0.1 Hz. After final placement of the electrodes, the skull opening was covered with a saline-agar mixture.

The hippocampal EEG signal was amplified on a high-gain AC preamplifier and displayed on an oscilloscope (Tektronix, Beaverton, OR). The fEPSP signal was recorded by a Macintosh 7100 Power PC computer (Apple Computer, Cupertino, CA) through an ITC Computer Interface (Instrutech, Great Neck, NY). The fEPSP was displayed on the computer monitor, and the fEPSP amplitude was measured using A/Dvance software (McKellar Designs, Vancouver, BC, Canada).

Hypoxia-ischemia. In experiments where unilateral fEPSP recordings were measured, the permanent carotid occlusion was coupled with a transient, 2-min exposure to moderate hypoxia. With the recording of simultaneous bilateral fEPSPs, the protocol was modified by coupling the vascular occlusion with little or no effect on the contralateral fEPSP. The hypoxic levels required to achieve this response ranged between 8 and 12% O₂, but a 10% O₂ hypoxic level was sufficient in most animals. Once the optimum level of hypoxia was established using the above criteria, it was left unchanged for subsequent hypoxic exposures.

Blood gas measurements. Arterial pH, PCO₂, and PO₂ were measured at 37°C with a Radiometer Copenhagen Blood Microsystem (BMS 3MK2) and a pH/blood gas monitor (pHm73). The electrodes were calibrated before and after each measurement. The pH electrode was calibrated with standard Radiometer buffers with pH values of 7.388 and 6.841 at 37°C. The PCO₂ and PO₂ electrodes were calibrated with Radiometer-certified calibration gases. The body temperature was measured with a thermistor and maintained at ~37°C with heating pads. Blood samples for hypoxic measurements were drawn during the fEPSP depression. Recovery blood samples were taken 5 min after the end of the hypoxic period. The blood gases were corrected for differences between the animal body temperature and 37°C with the appropriate correction factors for blood.

Cardiovascular parameters. In a separate group of experiments, heart rate (HR), blood pressure, and respiratory rate were recorded under the same experimental conditions; however, in these animals, electrophysiological recordings of the hippocampus were not taken. A lead II electrocardiogram was recorded using stainless steel needles placed subcutaneously as electrodes. The HR was determined from the R-R intervals. Blood pressure was recorded using a Gould P23 transducer, and tracheal airway pressure was monitored continuously using a differential pressure transducer via a small tube inserted into the tracheal cannula. All measurements were recorded using a Grass model 7P polygraph.

Agonist and antagonist application. 8-Cyclopentyltheophylline (8-CPT, 2.5 mg/kg, Sigma, St. Louis, MO) was dissolved in 2-hydroxypropyl-β-cyclodextrin (45% wt/vol, RBI, Natick, MA) and injected intraperitoneally. Trials with this solvent alone did not alter either the normoxic amplitude or the hypoxic depression of the fEPSP (n = 3). Caffeine (50 mg/kg, Sigma) was dissolved in saline and administered intraperitoneally.

Data analysis. Data were statistically analyzed using either a Student’s paired t-test or a one-way ANOVA followed by Student-Neuman-Keuls test for group comparisons. A P value of <0.05 indicated a significant difference.

![Fig. 1. Unilateral carotid artery occlusion coupled with moderate hypoxia results in a reversible depression of evoked synaptic transmission in the hippocampus ipsilateral to the occlusion (●, n = 8) but not contralateral to the occlusion (○, n = 7). The inspired oxygen was decreased to 10% to induce the 2-min period of hypoxia. The field excitatory postsynaptic potential (fEPSP) was recorded from either the ipsilateral or contralateral hippocampus in each animal. Recording of the fEPSPs was every 10 s, and the amplitude of each fEPSP was plotted as percentage of baseline amplitude. Means are plotted without SE to improve clarity of the figure. The fEPSP traces a, b, and c (inset) are representative waveforms for their respective times (●) recorded from the ipsilateral hippocampus.](http://ajpregu.physiology.org/DownloadedFrom)
RESULTS

Evoked synaptic potentials are reversibly inhibited by hypoxia. When attempts were made to induce a reversible inhibition of evoked synaptic transmission by coupling an acute common carotid artery occlusion with graded levels of hypoxia, it proved very difficult to obtain conditions that produced a consistent and reversible depression of the hippocampal fEPSP. More consistent responses were obtained by completing a permanent unilateral common carotid artery occlusion 1–2 h before exposure to hypoxic conditions. Subsequent recording and stimulation were performed in a single hippocampus either ipsilateral or contralateral to the occlusion. Under these conditions, it became apparent that the evoked potential recorded from the hippocampus ipsilateral to the carotid occlusion was more sensitive to the level of imposed hypoxia. A 2-min exposure to 10% O₂ consistently resulted in a reversible depression of the evoked fEPSP when recording from the hippocampus ipsilateral to the occlusion (Fig. 1). The depression of the fEPSP averaged 14.4 ± 2.9% of the prehypoxic amplitude (n = 8). After reintroduction of normoxia, the amplitude of the fEPSP recovered to 100.8 ± 2.4% of prehypoxic amplitude within a 5-min recovery period.

A second group of animals was given a permanent unilateral common carotid occlusion followed by stimulation and recording solely in the contralateral hippocampus. A 2-min hypoxic insult of 10% O₂ resulted in little change in the amplitude of evoked synaptic transmission at any time during or after the hypoxic insult (Fig. 1). During the hypoxic insult, the fEPSP amplitude remained at 99.4 ± 2.3% of the prehypoxic control amplitude (n = 7).

The reversible depression of evoked synaptic transmission in the ipsilateral hippocampus was repeatable, with no significant change in either sensitivity of the evoked potential to hypoxia or its recovery from hypoxia. Figure 2 illustrates that a series of five repeated exposures to hypoxia, each 2 min in duration with a 10-min recovery period between exposures, resulted in a reversible depression of synaptic transmission to within 5% of each other.

Responses to hypoxia are not affected by the side of stimulation. The differential sensitivity to hypoxia suggested the benefit of simultaneously recording from both hippocampi ipsilateral and contralateral to the side of occlusion. It was observed that stimulation of commissural fibers in the stratum radiatum of either hippocampus resulted in evoked potentials in both hippocampi. Stimulation intensity was adjusted to minimize any contamination of the fEPSP by population spikes. Under these conditions, the side of stimulation had no significant effect on the amplitude of the evoked potential in each hippocampus or in their indi-
individual responses to an hypoxic insult (Fig. 3). The normoxic fEPSP amplitudes recorded from the hippocampus ipsilateral to the occlusion measured $6.32 \pm 1.5\text{ mV (n = 8)}$ when the ipsilateral side was stimulated and measured $6.28 \pm 2.1\text{ mV (n = 8)}$ when the contralateral hippocampus was stimulated. The same consistency in normoxic fEPSP amplitudes was observed for the hippocampus contralateral to the occlusion. Furthermore, the depression in fEPSP amplitude, during a hypoxic period, was also shown to be independent of the side of stimulation. As shown in Fig. 3, the fEPSP ipsilateral to the occlusion was similarly depressed during hypoxia when either the ipsilateral or contralateral hippocampus was stimulated [compare Fig. 3A (top) with B (top)].

_Hypoxic depression of evoked potentials is proportionate to the level of hypoxia._ Figure 4 illustrates the proportionate responses to graded hypoxia and the differential sensitivity of the ipsilateral and contralateral hippocampus. Various levels of hypoxia (8, 10, and 12% O$_2$), each 2 min in duration, were given in one animal. The level of hypoxia could be adjusted to depress solely the ipsilateral response in a graded manner or could be increased sufficiently to reversibly depress evoked activity in both hippocampi. In this animal, the recovery of the fEPSP from the hippocampus ipsilateral to the occlusion was significantly delayed after 2 min of 8% O$_2$ compared with 2-min exposures to 10 and 12% O$_2$.

Fig. 4. The hypoxic depression of synaptic transmission is a graded response. The level of hypoxia could be adjusted to depress solely the ipsilateral response (top) in a graded manner or could be increased to depress the activity in both the ipsilateral and contralateral (bottom) hippocampus. Three levels of hypoxia, ranging from 8, 10, and 12% O$_2$, were given in one animal. Two-minute hypoxic periods at each level of hypoxia, with 10-min recovery periods between exposures, were used. Data are plotted as a percentage of prehypoxic amplitude at time 0.

Hypoxic depression of evoked potentials is proportionate to the level of hypoxia. Figure 4 illustrates the proportionate responses to graded hypoxia and the differential sensitivity of the ipsilateral and contralateral hippocampus. Various levels of hypoxia (8, 10, and 12% O$_2$), each 2 min in duration, were given in one animal. The level of hypoxia could be adjusted to depress solely the ipsilateral response in a graded manner or could be increased sufficiently to reversibly depress evoked activity in both hippocampi. In this animal, the recovery of the fEPSP from the hippocampus ipsilateral to the occlusion was significantly delayed after 2 min of 8% O$_2$ compared with 2-min exposures to 10 and 12% O$_2$.

Adenosine antagonists attenuate the hypoxic depression. Figure 5 illustrates the ability of adenosine antagonists to significantly attenuate the hypoxic depression of the fEPSP. With simultaneous, bilateral recordings, a level of hypoxia was chosen that produced the greatest depression of the ipsilateral fEPSP with little or no effect on the contralateral fEPSP. The drugs used were the nonselective adenosine antagonist caffeine and the adenosine A$_1$-subtype selective receptor antagonist 8-cyclopentyltheophylline (8-CPT) blocked the hypoxic depression of synaptic transmission. The effect of each drug on the hypoxic depression was examined 20 min after intraperitoneal drug administration. Neither drug altered the normoxic amplitude of the evoked potential. Caffeine (50 mg/kg, n = 7) and 8-CPT (2.5 mg/kg, n = 8) significantly attenuated the hypoxic depression of the fEPSP (*P < 0.05 compared with hypoxia alone).

Fig. 5. The nonselective adenosine antagonist caffeine and the adenosine A$_1$-subtype selective receptor antagonist 8-cyclopentyltheophylline (8-CPT) blocked the hypoxic depression of synaptic transmission. The effect of each drug on the hypoxic depression was examined 20 min after intraperitoneal drug administration. Neither drug altered the normoxic amplitude of the fEPSP. How-
ever, the depression of the evoked potential in response to a 2-min hypoxic insult was significantly attenuated by the administration of either drug. Hypoxia alone reduced the fEPSP to 14.4 ± 2.9% of control (n = 8). Caffeine (50 mg/kg ip) significantly blocked the hypoxic depression so that the fEPSP declined to 67.4 ± 5.7% of prehypoxic baseline amplitude (n = 7, P < 0.05). In the presence of 8-CPT (2.5 mg/kg ip), the hypoxic depression was also attenuated so that the fEPSP was reduced to 81.8 ± 5.8% of control (n = 8, P < 0.05).

**Attenuation of the hypoxic depression by 8-CPT wanes over time.** Figure 6 illustrates, from a single animal, that the effect of 8-CPT waned over time so that a return of the hypoxic depression could be recorded. Two initial hypoxic periods depressed the fEPSP to ~2% of the baseline amplitude. The injection of 8-CPT attenuated the hypoxic depression of the fEPSP to ~75% of the baseline amplitude. The depression in response to 10% O2 returned to predrug levels 3 h after the intraperitoneal injection of 8-CPT. In a group of animals, 3 h postdrug, a 2-min hypoxic challenge reduced fEPSP to 18.2 ± 3.1% of baseline amplitude (n = 8, data not shown). This sensitivity to hypoxia was not significantly different from the predrug control fEPSP at 14.4 ± 2.9% of baseline amplitude.

**Attenuation of the hypoxic depression with 8-CPT did not affect blood acid-base or cardiovascular responses.** In another group of animals (n = 5), it was shown that although 8-CPT administration was able to prevent the hypoxic depression of the fEPSP, it did not alter the blood acid-base responses to hypoxia. Blood gas measurements and electrophysiological data were simultaneously collected in these animals. Table 1 shows that hypoxia resulted in a significant decline in Po2 and PCO2 as well as a significant depression of the fEPSP amplitude. The administration of 8-CPT did not significantly alter normoxic blood gases or normoxic fEPSP amplitudes. Hypoxia, in the presence of 8-CPT, showed similar declines in Po2 and PCO2 despite the significant reduction in the depression of the fEPSP amplitude.

Finally, in a separate group of animals (n = 5), it was further shown that 8-CPT did not alter cardiovascular responses to hypoxia. Table 2 shows that hypoxia induced a significant decline in mean arterial pressure (MAP) with little change in HR. The administration of 8-CPT did not significantly alter normoxic MAP or HR, and in the presence of 8-CPT, hypoxia exhibited a similar decline in MAP.

**DISCUSSION**

We conclude from these studies that the initial hypoxic depression of hippocampal synaptic transmission observed in vivo is mediated by locally released adenosine acting at central A1 receptors. The adenosine antagonists caffeine and 8-CPT attenuated the hypoxic depression of synaptic transmission by 53 and 67%, respectively. Our observations with the selective antagonist 8-CPT are consistent with the numerous demonstrations of the role of the neuronal A1 receptor in inhibition of the in vitro hippocampal synaptic response during hypoxic or hypoxic-hypoglycemic conditions (1, 6, 7, 12, 16, 36).

It is unlikely that activation of other adenosine receptor subtypes directly contributes to the hypoxic depression of synaptic transmission. Activation of the A3 and A2a receptors facilitate synaptic transmission in the hippocampus primarily by negatively modulating

### Table 1. Arterial blood gas and electrophysiological measurements made before, during, and after hypoxia before and after drug administration

<table>
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<th>Control</th>
<th>Hypoxia</th>
<th>Recovery</th>
<th>Control</th>
<th>Hypoxia</th>
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<td>pH</td>
<td>7.46 ± 04</td>
<td>7.54 ± 03</td>
<td>7.47 ± 04</td>
<td>7.50 ± 05</td>
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<td>7.49 ± 04</td>
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<td></td>
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<td>PCO2, mmHg</td>
<td>34.3 ± 3.0</td>
<td>25.1 ± 1.8*</td>
<td>35.3 ± 1.7</td>
<td>30.1 ± 1.8</td>
<td>25.0 ± 2.3*</td>
<td>28.8 ± 2.4</td>
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<tr>
<td>PO2, mmHg</td>
<td>77.8 ± 12.2</td>
<td>32.6 ± 0.8*</td>
<td>83.2 ± 11.3</td>
<td>86.2 ± 13.3</td>
<td>36.6 ± 1.6*</td>
<td>85.8 ± 12.4</td>
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<td>[HCO3-], meq/l</td>
<td>22.3 ± 3.4</td>
<td>19.8 ± 3.0</td>
<td>22.3 ± 2.4</td>
<td>22.9 ± 2.4</td>
<td>21.1 ± 2.3</td>
<td>21.9 ± 2.7</td>
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<td>fEPSP, %baseline amplitude</td>
<td>100</td>
<td>4.43 ± 1.7*</td>
<td>101.0 ± 0.3</td>
<td>102.2 ± 0.9</td>
<td>82.53 ± 4.4†</td>
<td>102.8 ± 1.7</td>
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</table>

Values are means ± SE; n = 5 for all experimental conditions. Brackets, concentration; fEPSP, field excitatory postsynaptic potentials; 8-CPT, 8-cyclopentyltheophylline. *Significant difference from the control condition (ANOVA followed by Student-Neuman-Keuls). †Significant difference from no drug, hypoxic condition (ANOVA followed by Student-Neuman-Keuls).

### Table 2. MAP and HR measurements made before, during, and after 2-min hypoxic conditions either with no drug or after the administration of the adenosine A1 selective antagonist 8-CPT

<table>
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<th>Hypoxia</th>
<th>Recovery</th>
<th>Control</th>
<th>Hypoxia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>95.0 ± 4.1</td>
<td>36.8 ± 4.1*</td>
<td>97.7 ± 4.7</td>
<td>95.3 ± 3.1</td>
<td>40.2 ± 2.5*</td>
<td>96.5 ± 4.0</td>
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<td>HR, beats/min</td>
<td>399 ± 9.8</td>
<td>381.4 ± 4.7</td>
<td>402.0 ± 10.0</td>
<td>413.7 ± 9.0</td>
<td>406.0 ± 8.3</td>
<td>406.5 ± 7.8</td>
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</table>

Values are means ± SE; n = 5 for all experimental conditions. HR, heart rate. *Significant difference from the control condition (ANOVA followed by Student-Neuman-Keuls). The declines in mean arterial pressure (MAP) when compared with the no drug and + 8-CPT hypoxic conditions were not significantly different in magnitude (Student’s paired t-test).
A$_3$-mediated inhibition (5, 23). The activation of these receptors would oppose the hypoxic depression we observe. It is unlikely these receptors are substantially activated during the brief hypoxic-ischemic exposures applied in this study as both receptors appear to require an extended duration of activation and/or relatively high concentration of adenosine before antagonizing A$_3$-mediated inhibition (5, 17).

Under ischemia-like conditions, adenosine is released from hippocampus as adenosine rather than converted extracellularly from a nucleotide (22). The source of adenosine seems to be AMP dephosphorylation (19). However, it is difficult to quantitatively correlate adenosine outflow with nucleotide degradation because the adenosine efflux represents only a small fraction of the total nucleotide pool (19).

Adenosine is most likely released locally within the hippocampus. It is unlikely that the released adenosine is of peripheral origin given its half-life of <2 s in blood (28) and its inability to cross the blood-brain barrier (2, 3). Locally active adenosine is consistent with its rapid appearance in cerebral cortex within seconds of the onset of systemic hypoxia (35). There are several possible sources for the local production of adenosine. Adenosine pools exist in neurons, astrocytes, and endothelial cells. The adenosine originating from endothelial cells is unlikely to have access to neuronal receptors because it appears to be confined to the vascular side of the blood-brain barrier (3). However, both astrocytes and neurons exhibit vigorous transport and metabolism of adenosine (14, 25).

The most proximate stimulus for adenosine release seems to be local tissue hypoxia. Brain adenosine levels are sensitive to a number of factors that contribute to the level of tissue oxygenation including cerebral blood flow (CBF), MAP, and arterial PO$_2$ (24, 30, 35). With respect to this model, unilateral carotid occlusion in the absence of hypoxia should have had little effect on CBF, energy state, or cerebral metabolism (32). In this study, applied hypoxia resulted in combined hypotension and hypoxemia. Application of either one of these insults could result in reduced tissue oxygenation and metabolic derangement most prominently on the side ipsilateral to the carotid occlusion, with the hippocampus being one of the more vulnerable regions (11, 26, 32).

Although local tissue hypoxia may be the stimulus for adenosine release, it seems clear that the adenosine antagonists did not blunt the depression of the evoked potential by altering tissue oxygenation. The adenosine A$_1$ receptor selective antagonist 8-CPT blocked the hypoxic depression of synaptic transmission but did not change the hypoxic alterations in MAP, PO$_2$, and PCO$_2$. The persistence of synaptic transmission during hypoxia in the presence of 8-CPT is consistent with the conclusion that the adenosine-mediated inhibition of synaptic transmission is a physiological compensatory response to metabolic stress before compromise of function from energy depletion.

**Perspectives**

Numerous features of the adenosine-mediated depression of synaptic transmission are consistent with it being a physiological regulatory mechanism balancing energy supply and demand. Particularly appealing is the suppression of synaptic activity before neural function is compromised by energy depletion. Thus this adaptive strategy appears designed to maintain metabolic integrity at the expense of neural function. Possible adenosine-mediated behavioral correlates of this strategy would include fainting and restorative sleep.

It is not clear whether adenosinergic modulation functions to provide a continuous and proportionate adjustment of neural activity in response to varying energy supply or whether it acts as a “retaliatory” response to a relatively modest but critical threshold of energy compromise. Although evidence exists for a basal adenosinergic “tone,” we did not observe the expected effects of blocking this tone with the administration of antagonists. Under either scenario, it is an open question as to how adenosinergic inhibition of neural activity is integrated with regional autoregulation of CBF. Adenosinergic modulation of neural activity could be coupled to ongoing autoregulation to effectively maintain a balance of energy supply and demand. Or adenosinergic inhibition of synaptic transmission may signal the failure of cerebrovascular autoregulation. Finally, the brain is a somewhat unique organ in that excessive expression of its “function,” namely neurotransmitter release, can result in excitotoxic injury. Inhibitory modulation of synaptic function by endogenous adenosine as observed in this study should logically contribute to adenosine’s well-known actions as an endogenous neuroprotectant during ischemia.

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