Pharmacological Protection of Synaptic Function, Spatial Learning, and Memory from Transient Hypoxia in Rats

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ABSTRACT

Hypoxia significantly reduced cholinergic $\theta$ activity in rat CA1 field and intracellular $\theta$ in the CA1 pyramidal cells, recorded in hippocampal slices. The hypoxic responses of the hippocampal CA1 pyramidal cells to a brief hypoxia consisted of a short period of "synaptic arrest", observed as an elimination of excitatory postsynaptic current under voltage clamp and recovered immediately as oxygenation was reinitiated. The hypoxic synaptic arrest was not associated with reduced postsynaptic responses of the pyramidal cells to externally applied L-glutamate, suggesting that the synaptic arrest might result from a presynaptic mechanism. The hypoxic synaptic arrest was abolished in the presence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a specific adenosine $A_1$ receptor antagonist. Blocking adenosine $A_1$ receptors also eliminated effects of hypoxia on the hippocampal CA1 field $\theta$ activity and intracellular $\theta$ of the CA1 pyramidal cells. In behaving rats, brief hypoxia impaired their water maze performance in both the escape latency and probe tests. The impairment was prevented by intralateral cerebroventricular injections of DPCPX. These results suggest that hypoxia releases adenosine and produces an inhibition of synaptic transmission and intracellular signal cascade(s) involved in generation/maintenance of hippocampal CA1 $\theta$ activity. This protection of synaptic efficacy and spatial learning through adenosine $A_1$ receptor antagonism may represent an effective therapeutic strategy to eliminate functional interruption due to transient hypoxic episodes and/or chronic hypoxia secondary to compromise of respiratory function.

Hypoxia/ischemic stroke remains one of the most devastating threats to humans (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996; Lipton, 1999; Newman et al., 2001) and a big challenge to neuropharmacologists. Although all mammalian cells can sense and will respond to hypoxia (Eu et al., 2000), hippocampal CA1 pyramidal cells are among those, if not the, most sensitive to hypoxic/ischemic damage. In humans and other species including rats, the hippocampus has a broad role in information processing associated with memory, including spatial, declarative/relational, and episodic types of memory (Zola-Morgan et al., 1986). Many of the pyramidal cells are "place cells" (Shen et al., 1997) that fire whenever the animal is in a particular location in its environment (Muller et al., 1987) or when it receives a specific stimulus or performs a specific behavior in a particular place (Nadel, 1991). Thus, a selective deficit in explicit memory functions is associated with neuronal loss/damage largely restricted to the CA1 region of the hippocampus (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996).

Because of the extreme sensitivity of neural structures involved in memory, especially the hippocampal CA1 pyramidal cells, to hypoxia and ischemia, memory impairment is common after cerebral hypoxia/ischemia, bypass surgery, or heart attack (Grubb et al., 1996; Newman et al., 2001). Cognitive decline is evident in more than half to as many as three-quarters of patients at the time of discharge from hospitals after coronary-artery bypass grafting (Newman et al., 2001) as well as in patients with chronic lung diseases (Bianchi et al., 1986; Incalzi et al., 1997) or oropharyngeal abnormality (Horner et al., 1994). Hypoxic/ischemic consequences consist mainly of three forms: functional disruption, cellular injury, and delayed cell loss through apoptosis or necrosis, depending on the severity of the insult. Each form has a distinct pathophysiological characterization and calls for different therapeutics. In this study, we examined effects of transient hypoxia, mainly a functional interruption, on rat hippocampal CA1 synaptic plasticity and spatial memory. Brief hypoxia without obvious cell injury impaired the synaptic plasticity and ability of rats to master the water maze task. Spatial learning and functional impairment of the hippocampal CA1 synaptic plasticity are preventable by the adenosine $A_1$ receptor antagonist, DPCPX. The seriousness of this cognitive decline due to transient mild ischemia/hypoxia is indicated by the high incidence of cognitive impairment in patients at discharge after coronary-artery bypass surgery. More importantly, the memory decline is not tran-
sient and patients whose cognitive function decreases at discharge are at increased risk for long-term cognitive deficit and a reduced level of overall cognitive function (Newman et al., 2001), although it remains to be seen in what way the synaptic and memory impairments induced here are related to long-term cognitive damage in patients. Pharmacological prevention of the transient hypoxia/ischemia-induced impairment of synaptic plasticity and learning and memory, therefore, has important therapeutic value in reducing the risk for long-term cognitive decline.

Materials and Methods

Chemicals. Agents were perfused through the perfusion medium: kynurenic acid, carbachol, citocline, DPCPX, and atropine sulfat. All were purchased from Sigma Chemical Co. (St. Louis, MO). For in vivo evaluation of effects on spatial memory, DPCPX or vehicle was injected into the lateral cerebral ventricle through chronically placed cannulas.

Hippocampal Slice Electrophysiology. Male Wistar rats (150–200 g) were anesthetized with pentobarbital (60 mg/kg, i.p.), and the brains were removed and cooled rapidly in artificial cerebrospinal fluid (aCSF) solution (–4°C) bubbled continuously with 95% O2 and 5% CO2. Hippocampi were sliced (400 μm), placed in oxygenated aCSF solution (124 mM NaCl, 3 mM KCl, 1.3 mM MgSO4, 2.4 mM CaCl2, 26 mM NaHCO3, 1.25 mM NaH2PO4, and 10 mM glucose, pH 7.4), and perfused (2 ml/min) with the oxygenated aCSF in an interface chamber. The chamber was closed up by covering it with a removable plate, and only a small slit remained open for access of the electrodes to the tissue. Warmed, moist 95% O2/5% CO2 was blown over on top of the slices.

The CA1 pyramidal cells were recorded at 30–31°C with sharp electrodes (3 M KAc; tip resistance: 60–120 MΩ) except that in a few experiments, the bath temperature was raised to 37°C, as indicated otherwise in the text. Stable Schaffer collateral pathway (Sch)-CA1 excitatory postsynaptic responses (EPSPs) were evoked for several hours without noticeable change in EPSP amplitudes. Studies were performed on CA1 pyramidal cells with stable resting membrane potential more negative than –70 mV. These pyramidal cells were identified by their obvious accommodation, an identifying characteristic of pyramidal cells. Labeling the recorded cells exhibiting this characteristic with dye has previously revealed that the recorded cells are indeed pyramidal cells (Sun et al., 1999). Signals were amplified with an AxoClamp-2B amplifier and digitized and stored using DigiData 1200 with the P-Clamp data collection and analysis software (Axon Instruments, Inc., Foster City, CA). CAPACITANCE was optimally adjusted during discontinuous current-clamp mode before and after cell penetration to neutralize capacitance and reduce overshoot/undershoot errors as monitored on a second oscilloscope. Discontinuous single-electrode voltage-clamp mode was used for voltage clamping, employing a sampling rate of 3.0 to 5.0 kHz (30% duty cycle). Gain was usually set at 6 to 8 nA·mV−1, slightly below the maximum value without causing overshoot or instability in the step response to a repetitive 10-mV step command.

CA1 field potentials were recorded with glass microelectrodes filled with aCSF. Frequency and amplitude values of oscillation were taken from an average of five consecutive traces, all triggered at the same level of the same phase.

Bipolar stimulating electrodes (Teflon-insulated PtIr wire, 25 μm in diameter; FHC Inc., Bowdoinham, ME) were placed in the Stratum radiatum to stimulate Sch (20–40 μA, 50 μS), within 200 μm from the recording electrode. The intensity selected for stimulating the Sch-CA1 in each cell was about 60% below the intensity at which threshold EPSPs were elicited in initiation of action potentials in that cell. Test stimuli were applied at 1/min (0.017 Hz). High-frequency (100 Hz for 1 s) stimulation at the same intensity was used to induce long-term potentiation (LTP) of the glutamatergic EPSPs.

The initial slopes of the evoked EPSPs were analyzed and compared in evaluation of LTP. The average slope during a 10-min control period was taken as 100% for each individual cell. Experiments in which >20% variations in the evoked EPSP magnitudes occurred during the 10-min control period were discarded.

Spatial Maze Tasks. Effects of brief hypoxia and agents on spatial memory were evaluated in rats in vivo with the Morris water maze task. Male adult Wistar rats were housed in a temperature-controlled (20–24°C) room for a week, allowed free access to food and water, and kept on a 12-h light/dark cycle. Rats (200–225 g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p) and placed in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). The core temperature of rats was monitored and kept constant (38.0 ± 0.5°C) with a warming light and pad. Two stainless steel guide cannulas were placed bilaterally with the tips positioned at the coordinates (anterior-posterior, 0.5 mm; lateral, 1.5 mm; horizontal, 3.2 mm), under aseptic conditions. At the end of surgery and under appropriate anesthesia, rats received (s.c.) banamine (1 mg/kg) and ketoprofen (5 mg/kg) in a lactate-Ringer solution. A 7-day recovery period was allowed before any further experimentation.

All rats were randomly assigned to different groups (10 each) and swam for 2 min in a 1.5-m (diameter) × 0.6-m (depth) pool (22 ± 1°C). On the following day, rats were trained in a four trial/day task for 3 consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from the water by finding a fixed platform that was placed at a fixed location and submerged about 1 cm below the water surface. The navigation of the rats was tracked by a video camera. The escape latency and the route of rats swimming across the pool to the platform were recorded. The quadrant test (1 min) was performed after removing the platform, 24 h after the last training trial.

Hypoxia. Episodes of hypoxia (Sun and Reis, 1994) in vitro were induced by replacing the aCSF supply bubbled with 95% O2/5% CO2 with that bubbled with 90% N2/5% O2/5% CO2 for 3 min or 95% N2/5% O2/5% CO2 for 100 s and warmed, moist 90% N2/5% O2/5% CO2 or 95% N2/5% CO2, respectively, on top of the slices for the hypoxic period. The hypoxia in vitro was induced a half-hour after the induction of either Sch glutamatergic LTP or cholinergic θ and is milder than those used by others to produce an irreversible impairment of synaptic transmission. The same time frame (memory training and hypoxia at half-hour interval) was followed in vivo in the water maze spatial task (Fig. 1).

Brief hypoxia in vivo was induced by placing rats in a glass jar and

![Fig. 1. Time chart of experimental protocol in vivo. Before the spatial water maze trials, rats swam in the pool for 2 min (S). The training days consisted of 4 trials/day, 2 i.e.v. injections (between the first two and second two trials). One hundred seconds of hypoxia (N2 in a glass jar) were induced half an hour after the second and fourth trial of the day. Control rats were placed in the same jar for the same period (Air). The same procedure was repeated for 2 more days (×2) for each group and followed by the quadrant test (Q) that was performed 24 h after the last trial.](image-url)
Hypoxia Reduced Cholinergic $\theta$ Activity in the Hippocampal CA1. Hippocampal CA1 pyramidal cells were recorded in brain slices in vitro. Effects of brief hypoxia were monitored on synaptic transmission, LTP of glutamatergic EPSPs, and cholinergic $\theta$, a memory-related neuronal activity synchronization that appears to depend on hetrosynaptic interactions (Sun et al., 2001). Bath application of carbachol (50 $\mu$M, 20 min), a cholinergic receptor agonist, to hippocampal slices mimicked diffuse transmission by acetylcholine from septal activation (Descaries et al., 1997) and induced the CA1 $\theta$ field potential (Fig. 2b; at 7.4 $\pm$ 0.7 Hz from background noise, Fig. 2a; Table 1). The $\theta$ was sensitive to atropine blockade and lasted for more than 3 h, as reported by others (Huerta and Lisman, 1995). The $\theta$ oscillation of membrane potential (Table 1) was also observed in intracellular recordings from the hippocampal CA1 pyramidal cells (intracellular $\theta$; Fig. 2e, as compared with resting membrane potential trace before the $\theta$ induction, Fig. 2d).

Brief hypoxia (3 min of 5% $O_2$/5% $CO_2$/90% $N_2$), induced 30 min after $\theta$ induction, reduced $\theta$ activity by 87.4% (Fig. 2c and Table 1) and intracellular $\theta$ to a similar extent (Fig. 2f and Table 1). The reduction became evident near the end of the hypoxic period and lasted for longer than 1 h. While in control slices (without hypoxic challenge), no obvious changes in CA1 field $\theta$ (Table 1) or intracellular $\theta$ (Table 1) of the pyramidal cells were observed at the same time point when the hypoxic episode significantly reduced the $\theta$ activities.

The effects of hypoxia on cholinergic $\theta$ do not appear to be altered by raising temperature to 37°C. The cholinergic field $\theta$ magnitude (0.78 $\pm$ 0.04 mV; $n = 4$, $p < 0.05$) was significantly reduced (for longer than 1 h) by brief hypoxia (2 min of 5% $O_2$/5% $CO_2$/90% $N_2$, 30 min after $\theta$ induction) by 89.9% ($\pm$6.1%; $n = 4$, $p < 0.05$; 10 min after hypoxia).

**Hypoxia Did Not Affect Induced LTP.** Stimulation of Sch with a single pulse evoked an EPSP, which was stable for hours of intracellular recording and sensitive to blockade with kynurenic acid (500 $\mu$M, 20 min; not shown), a wide-spectrum glutamate receptor antagonist. High-frequency Sch stimulation (100 Hz, 1s) induced LTP of the Sch-CA1 EPSPs (Fig. 3, a and b). The hypoxic episode, except for inducing one

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**Table 1**

<table>
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<th>Tests</th>
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<th>Post-test</th>
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Fig. 2. Effects of brief hypoxia on hippocampal CA1 cholinergic CA1 $\theta$. Examples of recorded field potentials: precarbachol control (a), during carbachol (50 $\mu$M, 30 min; b) and 10 min after brief hypoxia (5% $O_2$, 3 min; c). Transient hypoxia dramatically reduced the cholinergic $\theta$ activity. Membrane potential traces of recorded CA1 pyramidal cells: precarbachol (d), during carbachol application (50 $\mu$M, 30 min; e), and 10 min after brief hypoxia (5% $O_2$, 3 min; f). The membrane potential was maintained at the precarbachol level by d.c. passing negative current (the second trace). The intracellular $\theta$ was also markedly reduced by the transient hypoxia.
point of synaptic arrest (see below), did not reduce induced Sch-CA1 LTP. The LTP was in fact slightly enhanced (Fig. 3, b and c), as compared with those without the hypoxic episode. The induced LTP was thus not vulnerable to the transient hypoxia at the time applied.

Hypoxia Produced a Synaptic Arrest without Affecting Postsynaptic Response to Glutamate. Hypoxia is known to block synaptic transmission of glutamatergic synaptic (Hammond et al., 1994), GABAergic (Rosen and Morris, 1993), and cholinergic synaptic transmission (Kása et al., 1997, Porkka-Heiskanen et al., 1997), causing disconnection, or synaptic arrest, of various neural circuits. These inputs and their interaction are known to play an essential role in enhancing synaptic efficacy in learning and memory (Shulz et al., 2000). Synaptic transmission in response to Sch activation was monitored with intracellular recordings from hippocampal CA1 pyramidal cells. Three minutes of hypoxia (5% O₂/5% CO₂/90% N₂) had no effect on the Sch-CA1 EPSPs or excitatory postsynaptic currents (EPSCs), except for the last minute, when the Sch-CA1 EPSPs and EPSCs were eliminated by the hypoxia (by 95.2 ± 5.6%, n = 10, and 96.8 ± 4.2%, n = 7, respectively, both p < 0.05; Fig. 4, a and b). This synaptic arrest immediately disappeared when reoxygenation was initiated (Fig. 4, a and b).

High levels of hypoxic insult are known to cause K⁺ release and produce a depolarization. However, the synaptic arrest induced here by minimal hypoxia could not result from neuronal depolarization, since no depolarization was observed during the entire brief period of hypoxia. The membrane potential was in fact hyperpolarized by 2 to 5 mV, which was overcome with a small depolarizing current to maintain the same membrane potential in each individual cell.

The role of a brief blockade of a postsynaptic response to Sch glutamatergic inputs during hypoxia was examined in seven CA1 pyramidal cells. The hypoxic synaptic arrest was found likely not to arise postsynaptically, since local application of L-glutamate during the last few seconds of the 3-min hypoxia revealed a peak inward current (201.2 ± 10.5 pA) that did not differ significantly (n = 7, p > 0.05) from their control values (206.8 ± 9.7 pA; Fig. 5, a and b). These results

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Fig. 3. Effects of brief hypoxia on long-term potentiation of hippocampal Sch-CA1 EPSPs. Representative Sch-CA1 EPSP traces (a) of post-high-frequency Sch stimulation (LTP, 40 min after the high-frequency stimulation) and pre-high-frequency Sch stimulation (Control). Representative Sch-CA1 EPSP traces (b) of pre-high-frequency Sch stimulation (Control), post-high-frequency Sch stimulation (LTP, 30 min after the high-frequency stimulation), and immediately after brief hypoxia (LTP-5% O₂ 3 min). c, time course of Sch-CA1 EPSPs in response to high-frequency Sch stimulation (at the first arrow) and brief hypoxia (at the second arrow; n = 10), compared with the control (n = 9) without hypoxic episode. The brief hypoxia produced a long-lasting period of enhancement of the established LTP. Data points are mean ± S.E.M. EPSPs were evoked 1-min⁻¹. For clarity, only every other point is shown. ■, control; ●, 5% O₂ for 3 min. The first sharp vertical lines were stimulation artifacts (10-ms delay).

Fig. 4. Transient hypoxia produced a brief period of synaptic arrest. Sch-CA1 EPSPs (a) and EPSCs (b) were briefly abolished at the end of brief hypoxia (5% O₂, 3 min), as compared with those of the next trace (recovery, 1 min a part) and of prehypoxia (Control). The first sharp vertical lines were stimulation artifacts (10-ms delay).
Effects of Adenosine A<sub>1</sub> Receptor Antagonist on the Hypoxic Synaptic Arrest. Hypoxia is known to cause the release of adenosine. The hypoxic synaptic arrest was prevented by blocking the adenosine A<sub>1</sub> receptors. In the presence of DPCPX, a selective adenosine A<sub>1</sub> receptor antagonist, the Sch-CA1 synaptic transmission remained intact at the end of the hypoxia (Fig. 6b, 99.2 ± 2.4% at the end of hypoxia versus control 100%; n = 7, p > 0.05). No synaptic arrest was observed in the presence of the antagonist in all the cases during the hypoxic period and posthypoxic recovery period (up to 1 h). Thus, the hypoxic synaptic arrest was eliminated rather than delayed by the adenosine A<sub>1</sub> receptor antagonist.

Application of citicoline, a neuroprotective substance (Shuaib et al., 2000), on the other hand, was ineffective (Fig. 6a; n = 6, p < 0.05), suggesting that certain, as yet unidentified, forms of cell damage do not appear to occur.

Fig. 6. Effects of adenosine A<sub>1</sub> receptor antagonist and citicoline on synaptic arrest elicited by transient hypoxia. In the presence of extracellular citicoline (100 μM, 30 min), the Sch-CA1 EPSP was eliminated at the end of the 3-min hypoxia (a). In the presence of DPCPX (10 μM, 30 min), however, synaptic arrest was abolished (b). The first sharp vertical lines were stimulation artifacts (10-ms delay).
revealed that DPCPX-hypoxia rats showed a preference for the target quadrant \( F_{3,36} = 169.7, p < 0.0001; \) Fig. 8e), identical to that of the control. The average swimming speeds for all 12 trials, however, did not differ between all the groups \( F_{11,312} = 50.14, p < 0.0001 \), and quadrant preference \( (c, d, \) and \( e) \), conducted at the end of the twelfth training session, and average swimming speed \( (\text{over 12 trials; } b) \). Rats \( (10 \text{ each}) \) were either subjected to air or hypoxia \( (95\% \text{ N}_2/5\% \text{ CO}_2 \text{ for 100 s}) \) in a glass jar, about 30 min after the second or fourth trial of the day. Bilateral i.c.v. DPCPX \( (400 \text{ nmol/site}) \) or vehicle was administered before the second and fourth trials of the day. Quadrant 4 is the target quadrant during training. **, \( p < 0.01 \). NS, \( p > 0.05 \).
Lack of oxygen, long before any evidence of cellular damage, induced in the present study was, however, much milder than in the majority of past studies. Several observations support the notion that a functional interruption during the transient hypoxic episodes, rather than cell injury, is responsible for the observed impairment of synaptic plasticity and spatial learning memory. First, full recovery in membrane potential and synaptic transmission of the CA1 pyramidal cells was observed. No widespread depolarization was evoked. Second, histological analysis after transient hypoxic episodes revealed no evidence of cellular damage. Third, the ineffectiveness of citicoline (Shuaib et al., 2000) further suggests that certain, as yet unidentified, forms of cell damage do not appear to occur. Nevertheless, the functional interruption seems to be specific, since average swim speed was not affected. The brief hypoxic episodes impair the spatial memory without significant effects on motor ability and neural control of motor activity. The functional interference of the hippocampal CA1 synaptic transmission and synchronized activity by transient hypoxia may, however, be more relevant to brief ischemic/hypoxic episodes that occur as silent stroke, brief cardiac arrest, or bypass surgery (Newman et al., 2001).

One of the most consistent consequences of hypoxia/ischemia in humans and other mammals is a decline of memory and the ability to learn and acquire novel experience. A consistent deficit in the water maze spatial learning following global cerebral ischemia/hypoxia has been demonstrated (for instance, Block and Schwarz, 1998). Two factors dictate the extreme sensitivity of explicit memory to cerebral hypoxia/ischemia: 1) an essential role of hippocampal neurons and networks (Alkon et al., 1998; Pearce et al., 1998; Riedel et al., 1999; Sun et al., 1999), particularly the hippocampal CA1 pyramidal neurons and synaptic inputs, for both encoding and retrieval of spatial memory and for either trace consolidation or long-term storage; and 2) the fragility of these networks to hypoxia-ischemia (Lipton, 1999). It is generally believed that it is the experience-induced modifications of synaptic strengths that enable the accumulation of a knowledge base. Loss of the relevant synaptic plasticity, as defined in the present study, may very well underlie the memory decline due to ischemic/hypoxic episodes. The extreme sensitivity of the hippocampal CA1 area versus other brain areas may be partially due to a high density of ionic channels and accelerated ultrastructural aberrations of capillaries in the CA1 in response to hypoxia/hypoperfusion (De Jong et al., 1999), although the study does not rule out the possibility that other regions of the hippocampus, or brain, may be rapidly affected by the hypoxic episodes and so contribute to the hypoxic memory impairment observed. Indeed, the synaptic plasticity was found to be extremely sensitive to the lack of oxygen, long before any evidence of cellular damage, consistent with others’ reports that factors other than the extent of CA1 cell loss contribute to behavioral impairments (Jaspers et al., 1990). Neuroprotective therapies have typically been evaluated in animal models simply by counting the remaining number of healthy cells or calculating infarct volume 1 to 7 days after the ischemic episode. In pathophysiological conditions such as were induced here, however, direct examination of protective effects on synaptic plasticity might be more important in the evaluation of potential therapies and/or preventive treatments.

Hypoxia-ischemia induces complicated responses of the brain (Lipton, 1999). Activities of neural groups (Sun and

Discussion

It is well established that functions of mammalian neurons are sensitive to acute hypoxia (Belousov et al., 1995; Lipton, 1999). The brain is a metabolically very active organ, but it contains virtually no O2 reserve. Upon a sudden occlusion of brain circulation (ischemia), the brain is left with an O2 content of about 0.2 ml/100 g and intracellular energy stores, which can support normal O2 consumption only for a few seconds and maintain cellular energy for 1 to 2 min at 37°C. Cerebral hypoxia/ischemia, as occurs with environmental limitations (at high altitude or in deep sea), insufficient blood flow (cerebrovascular hemorrhage, brain tumor, vascular occlusion, or cardiac arrest, bypass surgery), respiratory dysfunction (obstruction of airway, lung dysfunction, or neural control failure), or the use of some toxic substances, results in a high incidence of memory deficits and moderate-to-profound memory loss in humans (Grubb et al., 1996). Irreversible damage to brain tissue is caused by 10 min of severe hypoxia in vivo and in vitro (Lipton, 1999). The hypoxia

Fig. 9. Transient hypoxic episodes did not cause obvious cellular loss. Examples of Nissl-stained coronal sections of the dorsal CA1 field, revealing densely packed pyramidal cells with well-defined nuclei in control rats (a) and rats subjected to 8 episodes of brief hypoxia (b).
Reis, 1994, 1996) that control cardiovascular and respiratory systems, brain circulation, and other functions are also rapidly and powerfully affected. Hypoxia, depending on intensity and duration, modulates Ca\(^{2+}\), K\(^{+}\), Na\(^{+}\) channel activity and the expression of various genes and induces cellular injury, necrosis, and/or apoptosis (Lopez-Barneo et al., 1988; Sun and Reis, 1994; Hammarström and Gage, 2000). Hypoxia produces a synaptic arrest of glutamatergic (Hammond et al., 1994), GABAergic (Rosen and Morris, 1993; Hammond et al., 1994), and cholinergic synaptic transmission (Kása et al., 1997; Forkkka-Heiskanen et al., 1997). The effects of transient hypoxia on the synaptic transmission and plasticity of the hippocampal CA1 pyramidal cells is immediate and dramatic. So were effects of hypoxic episodes on spatial learning and memory. The functional interference of the synaptic transmission and synchronized rhythmic activity such as \(\theta\) by hypoxia may underlie these hypoxic effects. Spatial learning involves hippocampal \(\theta\). The firing and rhythmic activity of the pyramidal neurons depend on temporal interaction of cholinergic inputs and GABAergic inputs from interneurons, during behavior-related \(\theta\) activity. The difference between mechanisms underlying \(\theta\) and glutamatergic synaptic transmission may explain the prolonged \(\theta\) inhibition and short synaptic arrest of the glutamatergic synapse, induced by the same brief hypoxia in slices. The former involves temporal interaction of multiple synaptic inputs and cellular events. Similarly, the application of the adenosine A\(_1\) receptor antagonist most likely affected more than just the glutamatergic synaptic transmission, suggesting that adenosine is the main molecule that interferes with heterosynaptic interaction and produces the hypoxic \(\theta\) blockade. It remains to be examined whether the GABAergic and/or cholinergic synaptic responses are more sensitive to the brief hypoxic episodes. Although the present study focused on the short-term effects of brief hypoxia on \(\theta\) in vitro, a gradual recovery of the cholinergic \(\theta\) was observed when the recordings were kept long (\(\approx 2\) h) after the hypoxic episode. The time course and functional impact of the recovery also remain to be investigated. For instance, it is interesting to know whether the gradual recovery of cholinergic \(\theta\) after a hypoxic episode is essential for the remaining levels of spatial learning in hypoxic rats or whether the declined learning in the hypoxic rats involves a non-\(\theta\) compensating mechanism.

As far as we are aware, our study is the first demonstration that blocking adenosine A\(_1\) receptors prevents the impairment of spatial learning and memory and synaptic plasticity in response to noninjury hypoxic episodes. Our results show that glutamatergic EPSPs decreased near the end of the brief hypoxia. The decrease was apparently caused by adenosine release. Transient hypoxia/ischemia induces adenosine release (Van Wylen et al., 1986; Sun and Reis, 1994; Sun, 1996), resulting in opening of both K\(_{ATP}\) and K\(_{Ca}\) \(2^{+}\) channels, opposing responses involved in memory formation (Alkon et al., 1998), and decreasing stimulus-induced Ca\(^{2+}\) influx into neurons via actions at the presynaptic and postsynaptic adenosine A\(_1\) receptors. The involvement of adenosine A\(_1\) receptor activation is consistent with the observation that the hippocampal formation is highly enriched with the adenosine A\(_1\) receptors (Murphy and Snyder, 1982). The reduction in cholinergic \(\theta\) suggests an impaired heterosynaptic interaction, which is more complex than transmission at a single synapse. For stable \(\theta\) activity, some level of ongoing activity and interaction of heterosynaptic inputs may be necessary. In addition, adenosine A\(_1\) receptors are linked to G proteins and perhaps via these facilitate the opening of K\(^{+}\) channels. Internal Ca\(^{2+}\) release from an InsP\(_3\)-sensitive internal store might also be involved as a major consequence of hypoxic responses (Belousov et al., 1995). These mechanisms of hypoxia-induced pathophysiology, however, do not appear to involve inactivation of \(N\)-methyl-D-aspartate receptors, as reported in Western painted turtle cortical neurons (Bickler et al., 2000), a process that apparently involves activation of Ca\(^{2+}\)-dependent phosphatase(s) that may be critical for their remarkable ability to survive months without oxygen.

The hippocampal formation plays an important role in episodic, declarative, and spatial learning and memory and is an especially plastic and vulnerable brain structure that is damaged by hypoxia/ischemic stroke. Nevertheless, CA1 functional interference may underlie the observed spatial memory deficits due to transient hypoxia. LTP of Sch glutamatergic inputs, however, was not reduced but enhanced by the hypoxia. The hypoxic enhancement of the Sch-CA1 EPSPs due to the weak hypoxia applied in the present study was, in general, consistent with previously reported hypoxia-induced LTP (Hammond et al., 1994). Furthermore, our findings are consistent with previously reported observations that LTP expression is not vulnerable to transient hypoxia a few minutes later (Arai et al., 1990). It is also known that titanic LTP in the hippocampal CA1 could be readily induced minutes after anoxic episodes (Hammond et al., 1994). The slightly enhanced EPSPs and LTP, on the other hand, are unlikely to cause decreased spatial learning. Spatial learning has been reported to be normal with CA1 LTP that was enhanced 2-fold in inositol 1,4,5-triphosphate 3-kinase A-deficient mice (Jun et al., 1998). Nevertheless, episodes of transient hypoxia may be more relevant to a gradual memory decline during aging or Alzheimer’s disease (Gervais et al., 1999). The hypoxic synaptic arrest induced here compromises the ability of brains to learn and memorize. The value of preconditioning through adenosine A\(_1\) receptor activation needs careful evaluation, especially with transient hypoxia/ischemia. Relieving the network from heterosynaptic arrest through blocking the adenosine A\(_1\) receptors may represent an effective strategy to eliminate the functional impairment. Not only do such intervention strategies to reduce the perioperative cognitive decline have great value in reducing a late cognitive deterioration (Newman et al., 2001), but combined with agents that reverse cellular injury and prevent cell loss, the antagonists might also be valuable in therapy against severe hypoxia/ischemia-induced memory loss as well as progressive dementias such as Alzheimer’s disease.

References
De Jong GI, Parkas E, Stienstra CM, Plass JRM, Kreijer JN, De la Torre JC, and


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