Regulation of Excitatory Input to Inhibitory Interneurons of the Dentate Gyrus During Hypoxia

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Doherty, J. and R. Dingledine. Regulation of excitatory input to inhibitory interneurons of the dentate gyrus during hypoxia. J. Neurophysiol. 77: 393–404, 1997. The role of metabotropic glutamate receptors (mGlurs) and adenosine receptors in hypoxia-induced suppression of excitatory synaptic input to interneurons residing at the granule cell–hilus border in the dentate gyrus was investigated with the use of whole cell electrophysiological recording techniques in thin (250 μm) slices of immature rat hippocampus. Minimal stimulation evoked glutamatergic excitatory postsynaptic currents (EPSCs) in dentate interneurons in 68 ± 4% (mean ± SE) of trials during stimulation in the dentate granule cell layer (GCL) and 48 ± 3% of trials during stimulation in CA3. Hypoxic episodes, produced by switching the perfusing solution from 95% O2-5% CO2 to a solution containing 95% N2-5% CO2 for 3–5 min, rapidly and reversibly decreased the synaptic reliability, or probability of evoking an EPSC, from either input without reducing EPSC amplitude, consistent with a presynaptic suppression of transmitter release. The mGluR antagonist (+)-α-methyl-4-carboxyphenylglycine [(+)-MCPG; 500 μM] did not alter synaptic reliability or mean EPSC amplitude in either pathway. However, (+)-MCPG significantly attenuated hypoxic suppression of input from both pathways, suggesting that mGlurs activated by release of glutamate partially mediate hypoxic suppression of EPSCs to dentate interneurons. The mGluR agonist (15,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 100 μM) rapidly decreased the reliability of excitatory transmission from both the GCL (19 ± 5% of control) and CA3 (39 ± 15% of control). ACPD also increased the frequency of spontaneous EPSCs and evoked a slow inward current in dentate interneurons. Exogenous adenosine (10–300 μM) decreased synaptic reliability for both pathways and reduced the frequency of spontaneous EPSCs, but did not cause a decrease in the mean amplitude of evoked EPSCs, consistent with a presynaptic suppression of excitatory input to dentate interneurons. Conversely, the selective adenosine A1 receptor antagonists 8-cyclopentyl-1,3-dipropylxanthine (200 nM to 1 μM) and N6-cyclopentyl-9-methyladenine (1 μM) enhanced excitatory input to dentate interneurons by increasing synaptic reliability for both the GCL and CA3 inputs. Adenosine A1 receptor antagonists did not, however, reduce hypoxic suppression of excitatory input to dentate interneurons. These results indicate that hypoxia induces a presynaptic inhibition of excitatory input to dentate interneurons mediated in part by activation of mGlurs, but not adenosine A1 receptors, whereas both mGlurs and adenosine A1 receptors can depress excitatory input to dentate interneurons during normoxic stimulation. Regulation of excitatory input to dentate interneurons provides a mechanism to shape excitatory input to the hippocampus under both normal and pathological conditions.

INTRODUCTION

In the dentate gyrus, the activity of a heterogeneous group of GABAergic interneurons limits the firing of granule cells, which in turn provide a major excitatory input to hippocampal CA3 pyramidal cells (Han et al. 1993; Seress and Ribak 1990). Modulation of excitatory input to interneurons should therefore contribute significantly to the regulation of CA3 pyramidal cell excitability. We are interested in determining whether a decrease in the strength of excitatory input to inhibitory dentate interneurons contributes to the genesis of hyperexcitability that is observed in the hippocampus during epileptiform activity or in the initial stages of ischemia. We investigated whether hypoxia-induced suppression of synaptic input to pyramidal cells and interneurons of the CA1 region also occurs with excitatory synaptic inputs to dentate interneurons. Acute seizures sometimes follow ischemic episodes (Kilpatrick et al. 1990), suggesting that the suppression of inhibition that occurs during hypoxia may contribute to hyperexcitability.

Transient hypoxic episodes rapidly disrupt neuronal function, particularly in the hippocampus. Many studies suggest that different hippocampal subregions suffer varying levels of hypoxic-induced disruption (Cherubini et al. 1989; Kass and Lipton 1986; Kawasaki et al. 1990). For example, the granule cells of the dentate gyrus are more resistant to hypoxic disruption than are the pyramidal cells of the CA1 region (Aitken and Schiff 1986; Kass and Lipton 1986; Krnjevic and Ben-Ari 1989).

In the CA1 region, where the in vitro effects of hypoxia have been most extensively examined, brief hypoxic episodes profoundly suppress both excitatory and inhibitory synaptic inputs to pyramidal cells. Polysynaptic inhibition of CA1 pyramidal cells is more rapidly suppressed than excitation (Fujiwara et al. 1987; Hershkowitz et al. 1993), whereas monosynaptic inhibition is relatively resistant to hypoxia (Zhu and Krnjevic 1994). A similar hypoxic suppression of both excitatory postsynaptic currents (EPSCs) and polysynaptic inhibitory postsynaptic currents has been reported for input to stratum lacunosum–moleculare interneurons (Congar et al. 1995; Khazipov et al. 1993). Extracellular adenosine concentration rises rapidly after ischemic incidents (Pedata et al. 1993; Winn et al. 1981) and adenosine A1 receptors contribute to the hypoxic suppression of excitatory (Fowler 1990; Gribkoff et al. 1990; Katchman and Hershkowitz 1993; Zeng et al. 1992) but not inhibitory (Katchman and Hershkowitz 1993) input to CA1 pyramidal cells and excitatory input to CA1 interneurons (Khazipov et al. 1995). Adenosine can be generated extracellularly through the hydrolysis of released nucleotides by ecto-5′-nucleotidases or can be produced in the cytosol and transported to the extracellular space (see Higgins et al. 1994).
The concentration of glutamate in the extracellular space also rises during hypoxia (Lutz et al. 1994; Zini et al. 1993). Whereas the hypoxic release of glutamate contributes to ischemic damage through the activation of ionotropic glutamate receptors (reviewed by Szatowski and Attwell 1994), the potential role of metabotropic glutamate receptors (mGluRs) in the suppression of synaptic input to interneurons during hypoxia has not been addressed.

This study was designed to investigate the regulation of excitatory inputs to interneurons in the dentate gyrus by assessing the effects of brief hypoxic episodes on two different excitatory synaptic inputs to visually identified dentate interneurons with the use of whole cell patch-clamp recordings. We found that brief hypoxic episodes rapidly suppressed evoked excitatory input to dentate interneurons, probably through a presynaptic mechanism. This suppression was antagonized by (+)-α-methyl-4-carboxyphenylglycine [(+)-MCPG], an mGluR antagonist. Selective adenosine A1 receptor antagonists, however, failed to prevent hypoxic suppression of excitatory transmission. In contrast, adenosine A2 receptor antagonists, but not (+)-MCPG, suppressed excitatory input to dentate interneurons during normoxic conditions. These experiments indicate that hypoxic suppression of synaptic transmission occurs in dentate interneurons, but is mediated through different mechanisms than those reported for hypoxic suppression in CA1.

**METHODS**

Thin (250 μm) hippocampal slices were prepared from immature (10–16 day) male Sprague-Dawley rats. Slices were cut with a Vibratome (Lancer) in oxygenated (95% O2-5% CO2), ice-cold artificial cerebrospinal fluid (ACSF). Slices were transferred to a holding chamber and incubated at 30°C for ≥60 min, then transferred to a submerged recording chamber, immobilized with a nylon-mesh-covered platinum frame, and continuously perfused with room-temperature ACSF (composition, in mM: 130 NaCl, 3.5 KCl, 1.5 CaCl2·2 H2O, 1.5 MgSO4·7 H2O, 24 NaHCO3, 1.25 NaHPO4, and 10 glucose, pH 7.4, 295–305 mosM) at a rate of 2–3 ml/min. Transient hypoxic episodes were induced by switching the perfusion from normal ACSF to ACSF containing 95% N2-5% CO2, typically for 3–4 min, but in a few experiments for up to 8 min.

Whole cell patch recordings (Edwards et al. 1989) were performed with the use of an Axopatch 1D electrometer (Axon Instruments). Responses were filtered at 1–3 kHz with an eight-pole Bessel filter and digitized at 10–30 kHz on an IBM-compatible 80486 computer with the use of pClamp and Axotape data acquisition software (Axon Instruments). Patch electrodes (5–6 MΩ) were pulled from borosilicate glass with the use of a two-stage vertical puller and were filled with 130 mM CsOH, 140 mM methanesulfonic acid, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 2 mM MgCl2, and 1% biocytin. Intracellular solution was adjusted to pH 7.3 with CsOH and to 275–280 mosM with H2O. All experiments were performed at a holding potential of −70 mV in the presence of bicuculline methobromide (10 μM) unless otherwise noted. Seal formation and whole cell configuration were achieved in current-clamp mode, where passive membrane properties and action potential amplitudes were measured before switching to voltage-clamp mode.

Individual dentate interneurons were visually selected for electrophysiological investigation with the use of Hoffman modulation contrast optics (×600) on the basis of morphological criteria. There are at least six morphologically identified subtypes of GABAergic interneurons within the dentate gyrus (Han et al. 1993; Ribak and Seress 1983), each possessing different somal shapes and patterns of dendritic and axonal arborization. For example, pyramidal-shaped interneurons with somata located at the granule cell–hilus border have classically been termed basket cells on the basis of their axonal projections forming “basketlike” plexuses around individual dentate granule cells. Several morphological criteria were employed to identify inhibitory interneurons investigated for this study. The first criterion was a large soma at the base of the stratum granulosum near the transition to the hilus and a thick apical dendritic projection through the stratum granulosum into the stratum molecular, as described by Seress and Ribak (1983). The second criterion, the pattern of labeled axonal projections, was evaluated in biocytin-stained neurons.

Extracellular recordings were made from the stratum radiatum of CA1 with the use of ACSF-filled glass micropipettes (3–4 MΩ). Field potentials were evoked with monopolar tungsten microelectrodes placed in the stratum radiatum of CA3.

ACSF-filled glass pipettes were used to deliver electrical stimulation (0.3 Hz, 10–80 μA, 300–400 μs) to sites within the fascia dentata and area CA3. Stimulating pipettes were placed in the granule cell layer (GCL) 10–50 μm from the recording site or in the stratum pyramidale of the CA3b and CA3c regions. In experiments in which stimuli were delivered to both the GCL and CA3, the interpulse interval between stimuli at the two sites was 100 ms and the stimulus frequency was maintained at 0.3 Hz. Stimulus intensities were adjusted to the minimal level necessary to evoke visually identifiable EPSCs. Multiple sites were tested in each region to isolate a single reliable EPSC as well as to prevent antidromic activation of interneurons in response to stimulation in the stratum granulosum.

Evoked EPSCs were visually differentiated from synaptic failures with the use of software routines written in Microcal Origin. To be accepted for analysis, evoked events were required to meet several criteria. These included a short mean latency (2–7 ms) from the stimulus artifact, characteristic fast rising and exponential decay phases, and peak amplitudes that exceeded 2 SD of the mean amplitude of the baseline electrical noise. Events chosen for analysis were confined to a 2-ms window centered around the mean latency to minimize the possibility of counting spontaneous EPSCs as evoked events. Stimulus trials that did not produce events meeting these criteria were designated as synaptic failures and were not included in the calculation of EPSC parameters. The failure rate in synaptic transmission for each excitatory input was defined as the percentage of failures over the total number of stimulus trials. Individual inputs were not considered for further analysis if the mean failure rate was >90% during control stimulation. Transmission rate was defined as 100% − failure rate.

The minimum number of stimulus trials required to provide an accurate measurement of the transmission rate was determined by calculating the coefficient of variation of the transmission rate in 15 neurons when the sample size (number of consecutive stimuli) was systematically varied from 4 to 48. We found that the coefficient of variation reached a minimum with a sample size of 15 stimuli; however, for this study transmission rates were calculated on the basis of 20 consecutive stimuli.

Kinetic properties of both evoked and spontaneous EPSCs were analyzed with the use of the N05 software written by Dr. S. Traynelis. Rise times (10–90%) were fit by linear regression and decay time courses were fit to single exponentials with the use of a simplex algorithm. Data are expressed as means ± SE. Statistical significance was tested by either two-tailed Student’s t-test or by one-way analysis of variance followed by Bonferroni t-tests on selected pairs of data.

**Morphological evaluation**

To provide morphological characterization for each recorded neuron, individual slices were removed from the recording chamber
HYPOXIA DEPRESSES SYNAPTIC INPUT TO DENTATE INTERNEURONS

395

after physiological investigation and treated with 4% paraformaldehyde in phosphate-buffered saline for ±24 h. Some slices were subsequently treated with 30% sucrose in phosphate-buffered saline and resectioned at 50 µm on a cryostat microtome. Slices were treated with 10% methanol and 3% peroxide to inactivate native peroxidase activity and then permeabilized with 0.5% Triton X100 for 10–30 min. Slices were then exposed to an avidin–horseradish peroxidase solution (Vectorstain ABC kit, Vector Labs) with 3,3’-diaminobenzidine and hydrogen peroxide (0.02%) to induce a colorometric reaction. Stained slices were dehydrated in alcohol steps and mounted on glass slides with Permount (Fisher). Images of selected dentate interneurons were captured to an image analysis program (Image1, Universal Imaging) with the use of a charge-coupled device camera mounted on the microscope.

Drugs

Agents used were bicuculline methobromide (10 µM), 6-cyano-7-nitroquinolinaxine (3 µM), d(-)2-amino-5-phosphonopentanoic acid (50 µM), adenosine (10–300 µM), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.5–10 µM), N6-cyclopentyl-9-methyladenine (N-0840, 10 µM), (1S,3R)-1-aminoocyclcopentane-1,3-dicarboxylic acid (ACPD, 30–100 µM), and (+)-MCPCP (500 µM). All drugs except DPCPX and N-0840 were dissolved in ACSF and delivered by bath perfusion. DPCPX and N-0840 were initially dissolved in ethanol (final concentration 0.1%) and then diluted in ACSF. DPCPX and N-0840 were obtained from Research Biochemicals; all other drugs were obtained from Tocris Cookson.

RESULTS

Identification of dentate interneurons

Whole cell patch recordings were made from 79 visually identified dentate interneurons. Recordings from these cells had a series resistance of 14 ± 0.2 (SE) MΩ (n = 78), an input resistance of 290 ± 9.7 MΩ (n = 79), and a resting membrane potential of -50 ± 0.5 mV (n = 76). In five experiments in which potassium gluconate replaced cesium methanesulfonate in the patch solution, injection of current pulses in current-clamp mode generated nonaccommodating trains of action potentials.

Morphological analysis of biocytin stained neurons recovered from 35 experiments confirmed somal position at the stratum granulosum–hilus border. Dendritic spines were not observed on any stained interneurons. Examples of biocytin-stained dentate interneurons are shown in Fig. 1. Interneurons with pyramidal-shaped somata have recently been shown to have axonal projections into both the dentate molecular layer and the hilus, as well as within the GCL (Scharfman 1995; Soriano and Frotscher 1993), suggesting a functional diversity among dentate interneurons. The patterns of axonal projections recovered from interneurons in this study displayed significant diversity. A total of 7 interneurons possessed stained axons that projected solely to the GCL, 14 had axonal projections to both the GCL and the molecular layer (e.g., Fig. 1), 2 had axonal projections to the outer molecular layer, and no axonal projections were recovered from 12 stained neurons. The absence of axonal staining in any dentate region might be attributable to severing of axonal projections during the slicing procedure. Biocytin-labeled axonal projections could often be traced to the slice surface, indicating that axonal projections of dentate interneurons in vivo extend beyond the boundaries of the in vitro slice, as reported by Buckmaster and Schwartzkroin (1995). Despite the diversity of axonal projections observed in this study, no differences in either the electrophysiological characteristics of excitatory inputs or the responsiveness to hypoxic suppression of excitatory transmission were detected among the interneurons in this study, and therefore results from all recordings were pooled.

Evoked EPSCs

Dentate interneurons, basket cells in particular, receive excitatory synaptic input from dentate granule cells (Kneisler and Dingledine 1995a; Scharfman et al. 1990), the perforant path (Kneisler and Dingledine 1995a; Scharfman 1995; Scharfman and Schwartzkroin 1990), hilar interneurons (Scharfman 1994), and CA3 pyramidal cells (Kneisler and Dingledine 1995b). We examined excitatory input from both CA3 and dentate granule cells. The isolation of unitary EPSCs, evoked after transmitter release from a single release site, can provide valuable insights into the pre- or postsynaptic locus for the modulation of transmission at a given synapse. The goal of the minimal stimulation technique (Allen and Stevens 1994; Raastad et al. 1992) is to activate a single afferent axon in isolation from all other afferent fibers. This is achieved by lowering the stimulus intensity until the amplitudes of evoked and spontaneous EPSCs are similar, just above the threshold for activation. When minimal stimulation has been achieved, small changes in the stimulus intensity no longer alter the mean EPSC amplitude or the transmission rate, suggesting the activation of a single afferent (Fig. 2B). Minimal stimulation of either CA3 stratum pyramidale (Fig. 2A) or the stratum granulosum of the dentate gyrus evoked EPSCs in dentate interneurons in the presence of the γ-aminobutyric acid-A (GABA_A) receptor antagonist bicuculline methobromide (10 µM).

The positions of the stimulating electrodes and the intensity of the stimuli were optimized in each experiment to evoke short-latency, minimal-amplitude EPSCs. Minimal EPSCs from either pathway possessed kinetic properties indistinguishable from those of spontaneously occurring EPSCs, as summarized in Table 1. Stimulation of CA3 evoked EPSCs at a latency of 4.3 ± 0.2 ms (n = 25 cells) with an amplitude of -13.6 ± 0.8 pA (n = 25 cells). A second group of EPSCs, evoked after stimulation of CA3 at a latency of 13 ± 0.5 ms (n = 11 cells), had kinetic properties that were slower than those of the short-latency population of EPSCs (rise time 1.3 ± 0.2 ms; decay time constant 6.6 ± 1.5 ms). Considering their extremely long latency, it was assumed that these inputs probably resulted from polysynaptic activation of dentate interneurons; these responses were not included in this study. Stimulation of the stratum granulosum evoked EPSCs at a latency of 3.3 ± 0.2 ms (n = 25 cells) with an amplitude of -15.3 ± 1.4 pA (n = 20 cells). The mode of evoked EPSCs was somewhat smaller, ~10 pA. A combination of the ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinolinaxine (3 µM) and d(-)2-amino-5-phosphonopentanoic acid (50 µM) completely blocked EPSCs evoked from both pathways (data not shown), indicating that evoked EPSCs resulted from glutamatergic transmission, as previously reported by...
FIG. 1. Morphology of dentate interneurons. Insert: position of each interneuron in the dentate gyrus. A, top: camera lucida drawing of a biocytin-stained pyramidal basket cell of the dentate gyrus. Thick lines: dendritic arbors. Thin lines: axonal branches. Dotted lines: boundaries of the granule cell layer (GCL). The soma is situated at the border of the GCL and the hilus (HIL). A, bottom: photomicrograph of the same interneuron in a resectioned 50-µm tissue slice reveals the relative position of the dentate granule cells to the interneuron. Note that the thick apical dendrite passes completely through the GCL and enters the molecular layer (MOL) before bifurcation. Calibration bar: 25 µm. B, top: fusiform basket cell at the GCL-HIL boundary. Note the extensive axonal arborization in both the GCL and the MOL. B, bottom: segments of axon appear to encircle individual granule cells (arrow) in a photomicrograph of the same biocytin-stained cell in a 250-µm whole mount slice.

Kneisler and Dingledine (1995b). Decay time courses of both evoked and spontaneous EPSCs (see Table 1) compare well with the rapid desensitization times found for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in outside-out patches taken from dentate interneurons (Koh et al. 1995). Stimulation parameters were optimized to evoke EPSCs of unitary amplitude; however individual EPSCs of larger amplitude were observed in many experiments (e.g., Fig. 2C), presumably resulting from the activation of multiple release sites from either the same or different presynaptic fibers.

The differences in the mean amplitudes of evoked and spontaneous EPSCs, as well as the distribution of EPSC amplitudes (Fig. 3A), were not statistically significant (Table 1), suggesting that EPSCs evoked with minimal stimulation resulted from the activation of single, or at most a few, presynaptic release sites. Large-amplitude spontaneous EPSCs were sometimes observed; however, such large-amplitude events were not observed in the distributions of evoked EPSC amplitudes (Fig. 3A). The amplitude of the baseline noise (<5 pA) was measured during individual synaptic failures (Fig. 3A, inset). Amplitude distributions of evoked EPSCs did not significantly overlap the distribution of noise amplitudes, allowing EPSCs >5 pA to be reliably discriminated from synaptic failures.

**Stability of the transmission rate**

Synaptic transmission at both CA3-to-interneuron and GCL-to-dentate interneuron synapses has been reported to be unreliable (Kneisler and Dingledine 1995a,b). The transmission rates for both pathways, defined as the percentage of stimuli that evoked detectable EPSCs, was highly variable between different experiments (10–90%). Although excitatory transmission in each pathway was unreliable, input from the GCL was significantly (unpaired t-test; P < 0.05) more reliable than input from CA3. Electrical stimulation evoked minimal EPSCs in 68 ± 4% of stimulus trials follow-
ing stimulation in the stratum granulosum \((n = 25\) experiments), but only in 48 ± 3% of stimulus trials following stimulation in CA3 \((n = 35\) experiments). In five experiments, stimulating electrodes were placed in both CA3 and in the stratum granulosum. When stimulus trains were delivered to both CA3 and the stratum granulosum, transmission rates for EPSCs evoked from either CA3 input \((50 ± 5\%\) ) or stratum granulosum input \((71 ± 8\%\) ) were not significantly different from the transmission rates observed when only one pathway was stimulated, but were significantly different from each other (paired \(t\)-test; \(P < 0.05\) ).

Whereas the range of transmission rates observed from excitatory inputs was quite variable, the transmission rate of an individual excitatory input from either the stratum granulosum or CA3 pathway was stable in the absence of further manipulations (Figs. 2C and 3B).

Antidromic action potentials were evoked in whole cell recordings from four dentate granule cells and three CA3 pyramidal cells in response to stimulation in the dentate hilus or stratum radiatum of CA3, respectively. An increase in stimulus intensity of \(9 ± 2\) μA raised the probability of evoking antidromic action potentials from 0% to 50% when data were pooled from all cells.

### Effects of hypoxia on dentate basket cells

Brief hypoxic episodes rapidly and reversibly suppressed evoked excitatory input to dentate interneurons. An example of the effects of hypoxia on a CA3-evoked input to a dentate interneuron is shown in Fig. 4A. Suppression of synaptic transmission began within 1.5–2 min and peaked at 5–6 min after the start of a 3- to 4-min infusion with nitrogen bubbled ACSF (Fig. 4B). Recovery was rapid, with transmission rates recovering to control levels within 3–4 min from the end of the hypoxic episode (Fig. 4B). Each cell experienced only a single hypoxic episode.

Hypoxic episodes did not completely block evoked EPSCs in most experiments, instead sharply increasing the propor-

### TABLE 1. Kinetic properties of interneuron EPSCs

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<tr>
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<th>CA3-Evoked EPSCs</th>
<th>GCL-Evoked EPSCs</th>
<th>Spontaneous EPSCs</th>
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<tr>
<td></td>
<td>Control</td>
<td>With MCPG</td>
<td>With DPCPX</td>
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<tr>
<td>Amplitude, pA</td>
<td>(-13.6 ± 0.8) (25)</td>
<td>(-17.7 ± 8.5) (6)</td>
<td>(-13.6 ± 1.3) (7)</td>
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<tr>
<td>Latency, ms</td>
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<td>(4.3 ± 0.2) (6)</td>
<td>(4.2 ± 0.3) (7)</td>
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<tr>
<td>Rise time, ms</td>
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<td>0.9 ± 0.2 (6)</td>
<td>1.0 ± 0.2 (6)</td>
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<tr>
<td>Decay time constant, ms</td>
<td>3.6 ± 0.4 (20)</td>
<td>4.6 ± 0.5 (6)</td>
<td>3.3 ± 0.2 (7)</td>
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**FIG. 3.** Properties of evoked inputs to dentate interneurons. A: amplitude distributions of minimal evoked EPSCs (eEPSCs) mirror amplitude distributions for spontaneous EPSCs, suggesting that evoked responses represent unitary EPSCs. Distribution of noise amplitude, measured at the same latency during synaptic failures (inset), indicated that EPSC amplitudes >5 pA could be reliably resolved from baseline noise. B: each point represents the mean transmission rate for 20 stimuli. Results from 3 cells with CA3 input (○) and 3 with GCL input (•) are shown.

**FIG. 4.** Effect of a brief hypoxic episode on excitatory synaptic transmission. A: scatter plot depicting the effect of a brief hypoxic episode on CA3-evoked EPSCs in a dentate interneuron. Each point represents the result of 1 stimulus trial. B: plot of the effect of a 3-min period of hypoxia on the transmission rate of CA3-evoked EPSCs. Each point represents the value for transmission rate (mean ± SE) calculated from 12 different neurons and expressed as % of control transmission rate.
tion of stimuli that resulted in synaptic failure (Fig. 5A). Excitatory input was suppressed from both CA3 (29 ± 5% of control transmission rate, \( n = 25 \)) and the GCL (52 ± 8% of control transmission rate, \( n = 6 \)). Suppression of both inputs was observed when each was stimulated during the same hypoxic episode (Fig. 5B).

The mean amplitude of the less frequent EPSCs recorded during peak hypoxic suppression, evoked from stimulation of either CA3 (−13.0 ± 3.6 pA, \( n = 10 \)) or GCL (−17.5 ± 1.1 pA, \( n = 3 \)), was not significantly different from the means of EPSCs evoked during control stimulation of either input (Table 1). The consistency of EPSC amplitude suggests that the postsynaptic sensitivity to transmitter was not altered by brief hypoxia in dentate interneurons, as previously reported for CA1 neurons (Hershkowitz et al. 1993; Khazipov et al. 1995).

Hypoxic episodes could impact synaptic transmission through mechanisms other than a change in the probability of transmitter release. For example, hypoxia-induced decrease in extracellular volume could potentially limit the spread of stimulus current delivered to the axons of dentate granule cells or CA3 pyramidal cells, resulting in an axonal rather than transmission failure. Also, elevation of extracellular potassium levels could lower action potential initiation threshold, resulting in fewer failures. To assess axonal excitability in cells that synapse on dentate interneurons, we measured the percent change in the stimulus intensity needed to evoke antidromic action potentials in 50% of stimulus trials from both dentate granule and CA3 pyramidal cells. After a 5-min hypoxic episode, axonal excitability in four dentate granule cells and three CA3 pyramidal cells was unchanged (102 ± 6% of control; \( n = 7 \)), whereas orthodromic EPSCs evoked from both granule and pyramidal cells were suppressed by >90%. As the length of the hypoxic episode was extended to 10 min, the stimulus intensity needed to activate fibers progressively increased to 110 ± 8% of control (\( n = 5 \)), suggesting that a lowered axonal excitability could contribute to a depression of synaptic transmission during long hypoxic episodes. Our hypoxic episodes typically lasted <5 min, ending before significant changes in axonal excitability occurred.

In addition to the profound suppression of excitatory synaptic transmission, hypoxic episodes had several other effects on dentate interneurons, including an increase in the frequency of spontaneous EPSCs and, in a few cases, the appearance of repetitive bursts of EPSCs during both hypoxia and recovery. Hypoxic episodes failed to generate detectable slow currents in the majority (71%) of recordings from dentate interneurons in this study, although small hypoxic inward (−40.4 ± 12.1 pA, \( n = 8 \)) or outward (33.7 ± 18 pA, \( n = 5 \)) currents, or posthypoxic outward (13.9 ± 2.8 pA, \( n = 13 \)) currents were observed in some interneurons. Several factors may contribute to the absence of significant hypoxia-induced currents. Slow potassium conductances generated during hypoxia (Krnjevic and LeBlond 1989) would be attenuated in this study, because recordings were made with cesium-containing electrodes. Additionally, hypoxia-induced slow membrane currents in CA1 were observed to be smaller as a consequence of either the whole cell recording configuration (Zhang and Krnjevic 1993) or the use of slices from young animals (Cherubini et al. 1989) or at room temperature (Krnjevic and Walz 1990).

Role of mGluRs

Metabotropic receptors participate in the presynaptic suppression of transmitter release at several hippocampal synapses, including mossy fiber input to CA3 (Manzoni et al. 1995; Yoshino et al. 1996), Schaffer collateral input to CA1 (Baskys and Malenka 1991; Gereau and Conn 1995a; Vignes et al. 1995), and excitatory input to CA1 interneurons (Desai et al. 1994). To investigate the potential role of mGluRs during hypoxia in the dentate gyrus, we tested the effects of an mGluR antagonist, (+) MCPG, on hypoxic suppression of excitatory transmission in dentate interneurons. Pretreatment with (+) MCPG (500 μM), a selective antagonist for group I and II mGluRs, for 5–15 min had no significant effect on the mean transmission rate in normoxic conditions for EPSCs evoked from either CA3 [53 ± 6% in (+) MCPG vs. 47 ± 8% in control; \( n = 5 \)] or the GCL (88 ± 2% vs. 88 ± 3%; \( n = 3 \)). (+) MCPG (500 μM) also had no significant effect on the mean amplitude or kinetic parameters of CA3-evoked, GCL-evoked, or spontaneous EPSCs recorded from dentate interneurons (see Table 1). This suggests that (+) MCPG-sensitive mGluRs do not play a significant role in the modulation of synaptic transmission to inhibitory in-
Hypoxia depresses synaptic input to dentate interneurons

Although (+)MCPG had no apparent effects on evoked excitatory input, pretreatment (5–15 min) with 500 µM (+)MCPG significantly attenuated the hypoxic suppression of evoked EPSCs. Transmission rate of CA3 input decreased to 69 ± 8% of control after hypoxia in the presence of 500 µM (+)MCPG (Fig. 6, A and B; n = 5), compared with 29 ± 3% of control (n = 12) after hypoxia in the absence of (+)MCPG. The transmission rate during hypoxia plus (+)MCPG, however, remained significantly depressed from the control transmission recorded in the presence of (+)MCPG alone (paired t-test; P < 0.01). Thus 500 µM (+)MCPG provided significant (1-way analysis of variance; P < 0.001), but not complete, protection from hypoxic suppression of CA3 input to dentate interneurons. (+)MCPG at 500 µM also provided substantial protection from hypoxic suppression of GCL-evoked EPSCs (Fig. 6C; n = 4). The mean amplitudes of EPSCs evoked during peak hypoxic suppression of transmission in (+)MCPG [CA3 evoked: −17.7 ± 8.5 pA (n = 5); GCL evoked: −10.6 ± 2.5 pA (n = 3)] were not significantly different from control EPSC amplitudes (see Table 1).

Effects of ACPD on dentate interneurons

The attenuation of hypoxic suppression of excitatory transmission by (+)MCPG indicates that mGluR activation can regulate excitatory synaptic input to dentate interneurons. Therefore we tested the effect of ACPD on excitatory synaptic input to dentate interneurons. The mGluR agonist ACPD suppressed excitatory synaptic inputs from both CA3 (39 ± 15% of control transmission rate, n = 8) and the GCL (19 ± 5% of control transmission rate, n = 3) (Fig. 7A). The mean amplitude of evoked EPSCs in ACPD was not significantly different from control stimulation (paired t-test; P > 0.05). In eight experiments, the mean amplitude of CA3-evoked EPSCs was −17.3 ± 4.3 pA during control stimulation and −13.7 ± 8.8 pA in the presence of 100 µM ACPD. In three experiments, the mean amplitude of GCL-evoked EPSCs was −13.6 ± 4.7 pA during control stimulation and −11.2 ± 4.1 pA in the presence of 100 µM ACPD. Perfusion with ACPD (10–100 µM) increased both the frequency (Fig. 7B) and mean amplitude (Fig. 7C) of spontaneous EPSCs in dentate interneurons. A slowly developing inward current (−80.5 ± 26.1 pA) was observed in 8 of 11 interneurons treated with ACPD. These results, and those obtained with (+)MCPG, described above, support the idea that activation of mGlurRs contributes to presynaptic suppression of excitatory input to dentate interneurons during hypoxia.

Role of adenosine receptors

Adenosine A1 receptors have been demonstrated to mediate hypoxic suppression of excitatory transmission in CA1, both at Schaffer collateral synapses to pyramidal cells (Fowler 1990; Katchman and Hershkowitz 1993) and at inputs to lacunosum-moleculare interneurons (Khazipov et al. 1995). To determine whether adenosine receptors play a similar role for inputs to dentate interneurons, we studied the effects of both selective adenosine agonists and antagonists. The selective adenosine A1 receptor antagonist DPCPX (0.2–1 µM) significantly (paired t-test; P < 0.01) increased the rate of excitatory transmission in the CA3-evoked pathway from 21 ± 5% to 39 ± 10% in eight experiments, suggesting the presence of a tonic inhibitory effect of adenosine at these excitatory synapses onto dentate interneurons.

Surprisingly, however, pretreatment with the selective adenosine A1 receptor antagonists DPCPX (0.2–1 µM) or N-0840 (10 µM) for 10–60 min did not reduce hypoxia-induced suppression of excitatory synaptic input to dentate interneurons from CA3 (Fig. 8, A and B). Excitatory transmission in the CA3 pathway decreased to 36 ± 7% of control
EPSCs evoked during peak hypoxic suppression from CA3. The main conclusions that can be drawn from this study of excitatory transmission at Schaffer collaterals can be attributed to synaptic reliability, occurring through a presynaptic response to stimulation of the Schaffer collaterals (Fig. 8). Significantly attenuated hypoxic-induced suppression of field excitatory transmission under normoxic conditions. Agonists of the inability of DPCPX to block hypoxic suppression of tors. Both receptor types are present and functional at these dentate granule cells and thus increase excitatory mossy fiber still observed in the presence of DPCPX, suggesting that hypoxia causes presynaptic inhibition of excitatory inputs to inputs in the presence of DPCPX were not different from inputs from both CA3 and granule cells to dentate interneurons (Fig. 8B). Mean amplitudes of EPSCs evoked during peak hypoxic suppression from CA3 (−13.6 ± 1.3 pA) or stratum granulosum (−12.7 ± 4.3 pA) inputs in the presence of DPCPX were not different from EPSCs recorded before hypoxia (Table 1).

Brief hypoxic episodes suppressed synaptic input to CA1 pyramidal cells from the Schaffer collaterals during field potential recordings (Fig. 8C), as has been previously reported (Fowler 1990; Gribkoff et al. 1990). In contrast to the inability of DPCPX to block hypoxic suppression of excitatory input to interneurons in the dentate gyrus, DPCPX significantly attenuated hypoxic-induced suppression of field excitatory postsynaptic potentials recorded in CA1 in response to stimulation of the Schaffer collaterals (Fig. 8C). This suggests that adenosine-mediated hypoxic suppression of excitatory transmission at Schaffer collaterals can be attenuated by a concentration of DPCPX (1 μM) that is ineffective in attenuating hypoxic suppression of excitatory input to dentate interneurons. However, an initial suppression was still observed in the presence of DPCPX, suggesting that DPCPX may not completely block the earliest phase of hypoxic suppression of CA1 field EPSCs.

Exogenous adenosine (10–300 μM) profoundly decreased the mean transmission rate of both CA3 and GCL inputs. Perfusion with 100 μM adenosine decreased the transmission rate of CA3 input to 29 ± 2% of control transmission (Fig. 8D) (n = 4 cells) and that of GCL input to 47 ± 8% of control transmission (Fig. 8E) (n = 3 cells). This concentration of adenosine thus mimicked the magnitude of suppression of both inputs caused by hypoxia. In the presence of 1 μM DPCPX, inputs from both CA3 and the GCL were completely protected from adenosine-induced suppression (Fig. 8, D and E).

**DISCUSSION**

The main conclusions that can be drawn from this study are, first, that brief hypoxic episodes suppress excitatory inputs from both CA3 and granule cells to dentate interneurons in hippocampal slices from young rats; second, that the suppression of transmission to dentate interneurons by hypoxia is consistent with a presynaptic site of action; and third, that hypoxic suppression of excitatory input to dentate interneurons involves mGluRs, but not adenosine A1 receptors. Both receptor types are present and functional at these synapses, because selective agonists reversibly suppress synaptic transmission under normoxic conditions. Agonists of mGluRs or adenosine receptors do not, however, decrease the mean amplitudes of minimally evoked EPSCs, suggesting that the depression of transmission, indicated by lowered synaptic reliability, occurs through a presynaptic depression of transmitter release. Although postsynaptic effects cannot be completely ruled out (Edwards 1995), these results suggest that glutamate released in the early stages of hypoxia causes presynaptic inhibition of excitatory inputs to dentate interneurons, which in turn should tend to disinhibit dentate granule cells and thus increase excitatory mossy fiber input to CA3 pyramidal cells. This mechanism involving activation of mGluRs may therefore contribute to the hyperexcitability of the hippocampus that can be observed after ischemic or hypoxic episodes. Presynaptic suppression of excitatory input by mGluR activation may protect interneurons from excitotoxicity following transient hypoxia.
HYPOXIA DEPRESSES SYNAPTIC INPUT TO DENTATE INTERNEURONS

mGluRs suppress evoked EPSCs during hypoxia in dentate interneurons

We demonstrated that mGluRs participate in hypoxia-induced suppression of excitatory synaptic transmission to dentate interneurons. It has been reported (Opitz et al. 1994) that (+)MCPG increased the rate of recovery of field potentials in CA1 following a hypoxic episode; however, these data provide the first demonstration of the participation of mGluRs in the suppression of synaptic transmission during hypoxia. Because pretreatment with (+)MCPG at 500 μM provided near-complete protection of the GCL input from hypoxic suppression, but only partial protection of the CA3 input, it is not clear whether mGluRs are solely responsible for hypoxic suppression of synaptic transmission at the CA3 input.

mGluR antagonists had no discernible effect on excitatory transmission to dentate interneurons during low-frequency (0.3 Hz) electrical stimulation under normoxic conditions. A perisynaptic localization of mGluRs (Nusser et al. 1994) may explain the role of mGluRs in the suppression of hypoxic, but not normoxic, synaptic transmission if elevation of glutamate levels due to release from nonsynaptic sites or impaired transporter activity (see Diemer et al. 1993) is required for presynaptic mGluRs to be activated at excitatory synapses to interneurons in the dentate gyrus.

Excitatory inputs from both CA3 and the stratum granulosum were suppressed by 100 μM ACPD. mGluR agonists also suppress synaptic transmission at perforant path terminals (Brown and Reymann 1995; Harris and Cotman 1983), mossy fiber terminals (Manzoni et al. 1995), and Schaffer collaterals (Baskys and Malenka 1991; Gereau and Conn 1995a). mGluRs have been reported to suppress (Boss et al. 1992) or enhance (McBain et al. 1994) excitatory input to CA1 inhibitory interneurons. The suppression of input to dentate interneurons may contribute to the reduction of paired-pulse depression by mGluR agonists in the dentate gyrus (Brown and Reymann 1995). ACPD is a nonselective mGluR agonist, with affinity for both group I and II mGluRs (Roberts 1995). In situ hybridization indicates the presence of group I, II, and III mGluRs in CA3 pyramidal cells (reviewed in Testa et al. 1994). Identification of the mGluR receptors that contribute to suppression of excitatory transmission at
excitatory synapses onto dentate interneurons will require the use of more selective mGluR agonists.

As was the case with hypoxic suppression of excitatory input to dentate interneurons, ACPD did not reduce evoked EPSC amplitude. This suggests that the primary effect of ACPD on synaptic transmission at these synapses is a presynaptic suppression of transmitter release, which supports previous reports that used paired-pulse facilitation (Baskys and Malenka 1991) or analysis of miniature EPSC amplitudes (Gereau and Conn 1995a) to indicate a presynaptic effect of ACPD on synaptic transmission. Interestingly, in contrast to findings for interneurons of CA1 (McBain et al. 1994), we did not observe an increase in amplitude of evoked EPSCs in response to ACPD. We did, however, observe an increase in the mean amplitude and frequency of spontaneous EPSCs in dentate interneurons following application of ACPD, as previously reported (McBain et al. 1994), perhaps as a result of direct excitatory effects on pyramidal cells (Davies et al. 1995; Desai and Conn 1991; Gereau and Conn 1995a).

Adenosine A1 antagonists do not prevent hypoxic suppression of excitatory transmission in dentate interneurons

The inability of adenosine A1 receptor antagonists to prevent hypoxic suppression of excitatory input to dentate interneurons was surprising considering the role of A1 receptors in hypoxic suppression of synaptic input to both principal neurons (Fowler 1990; Gribkoff et al. 1990; Katchman and Hershkowitz 1993) and interneurons (Khniov et al. 1995) in CA1. Exogenously applied adenosine, acting through presynaptic adenosine A1 receptors, suppresses excitatory synaptic transmission at perforant path inputs to dentate granule cells (Prince and Stevens 1992), mossy fiber inputs to CA3 (Yamamoto et al. 1993), and Schaffer collaterals to CA1 (Lupica et al. 1992; Thompson et al. 1992; Wu and Saggau 1994).

Exogenously applied adenosine was effective in suppressing synaptic input to dentate interneurons in both of the pathways investigated in this study, and DPCPX increased the basal transmission rate of these inputs, indicating that A1 receptors are present at excitatory synapses or fibers (Swanson et al. 1995) from CA3 and dentate granule cells onto dentate interneurons. However, neither DPCPX (1 μM) nor N-0840 (10 μM) could reduce the hypoxic suppression of either input to dentate interneurons. It does not appear likely that a protective effect of the antagonist was overwhelmed by high concentrations of adenosine released at these synapses during hypoxia, because 1 μM DPCPX completely antagonized the suppression of excitatory transmission by 100 μM adenosine (Fig. 8, D and E), which produced the same degree of suppression of both inputs as did hypoxia. Basal levels of adenosine in extracellular fluid are reported to be 50–300 nM (Rudolph et al. 1992) and to become rapidly elevated after hypoxia-ischemia in whole brain (Rehncrona et al. 1978; Winn et al. 1981) or hypoxia in hippocampal slices (Pedata et al. 1993). Extracellular adenosine levels have been estimated to reach 2–40 μM during transient hypoxia (see Rudolph et al. 1992). These findings, taken together, suggest that, in contrast to interneurons in CA1 (Khazipov et al. 1995), adenosine levels at excitatory synapses on dentate interneurons are not sufficiently elevated during brief hypoxic episodes to suppress transmission. Evidence for regional differences in extracellular adenosine levels in the hippocampus is consistent with this suggestion. Adenosine agonists are more effective in inhibiting acetylcholine release in CA1 than the dentate gyrus, whereas DPCPX increases basal acetylcholine release in CA1, but not in the dentate gyrus (Cunha et al. 1994).

Activation of mGluRs can inactivate adenosine A1 receptors in cortical synaptosomes via a protein-kinase-C-dependent pathway (Budd and Nicholls 1995; Vasquez et al. 1995a), an effect seen preferentially in synaptosomes from young animals (Vasquez et al. 1995b). Thus mGluR regulation of inhibitory adenosine A1 receptors could also provide a mechanism for the apparent inactivity of A1 receptors receptors at terminals on dentate interneurons during transient hypoxia.

We have demonstrated that excitatory synaptic inputs to dentate interneurons can be rapidly disrupted by brief hypoxic episodes in hippocampal slices from young rats. This reversible suppression of transmission is consistent with a presynaptic site of action and is at least partially mediated by mGluRs, but not adenosine A1 receptors, although agonists of both types of receptors are capable of suppressing excitatory transmission at these synapses. The inability of adenosine A1 antagonists to prevent hypoxic suppression of excitatory transmission to dentate interneurons argues that some of the consequences of brief hypoxia in the dentate gyrus differ from those in CA1. Indeed, in contrast to CA1 and CA3 pyramidal cells, only weak hypoxia-induced currents are generated in dentate granule cells (Krnjevic and Ben-Ari 1989). Differential sensitivity of CA1 and the dentate gyrus to the effects of hypoxia has been reported in numerous studies (Aitken and Schiff 1986; Kass and Lipton 1986; Westgate et al. 1994).

It will be interesting to determine which mGluRs are responsible for suppression of excitatory transmission at these synapses, and whether the same mGluRs participate in the suppression of synaptic transmission at all inputs to interneurons. It will also be interesting to determine whether the inhibitory effect of mGluRs on the hypoxic suppression of transmission also occurs in adult animals or whether this effect is restricted to an early developmental stage. Finally, it will be important to determine whether the resulting disinhibition of dentate granule cells during hypoxia does indeed result in increased excitation of CA3 pyramidal cells, or whether this predicted effect is blunted by mGluR-mediated inhibition of mossy fiber transmission to CA3 pyramidal cells (Manzoni et al. 1995; Yoshino et al. 1996).
HYPOXIA DEPRESSES SYNAPTIC INPUT TO DENTATE INTERNEURONS


