Changes in the Calcium Dependence of Glutamate Transmission in the Hippocampal CA1 Region After Brief Hypoxia-Hypoglycemia

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OUANONOU, A. Y. ZHANG, AND L. ZHANG. Changes in the calcium dependence of glutamate transmission in the hippocampal CA1 region after brief hypoxia-hypoglycemia. J. Neurophysiol. 82: 1147–1155, 1999. Using the model of hypoxia-hypoglycemia (HH) in rat brain slices, we asked whether glutamate transmission is altered following a brief HH episode. The HH challenge was conducted by exposing slices to a glucose-free medium aerated with 95% N2-5% CO2 for ~4 min, and glutamate transmission in the hippocampal CA1 region was monitored at different post HH times. In slices examined ~8 h post HH, CA1 synaptic potentials are comparable in amplitude to controls, but are less sensitive to experimental manipulations designed to attenuate intracellular Ca2+ signals, as compared with controls. Reducing calcium influx, by applying a nonspecific calcium channel blocker Co2+ or lowering external Ca2+, attenuated CA1 synaptic potentials much less in challenged slices than in controls. Buffering intracellular Ca2+ by bis-(o-aminophenoxy)-N,N′,N′,N′-tetraacetic acid-AM (BAPTA-AM) attenuated CA1 synaptic potentials in control but not in slices post HH. Furthermore, minimally evoked excitatory postsynaptic currents displayed a lower failure rate in post-hypoxic CA1 neurons compared with controls. Based on these convergent observations, we suggest that evoked CA1 glutamate transmission is altered in the first several hours after brief hypoxia, likely resulting from alterations in intracellular Ca2+ homeostasis and/or Ca2+-dependent processes governing transmitter release.

INTRODUCTION

Alteration in glutamate synaptic activity is the key component of pathophysiology in ischemic brain damage (Luhmann 1996; Xu and Pulsinelli 1996). Brief exposure of brain slices to hypoxia produces dramatic changes in synaptic responses in the hippocampal CA1 region (Fujiwara et al. 1987; Hansen et al. 1982; Leblond and Krnjević 1989; Lobner and Lipton 1993). CA1 synaptic events mediated by ionotropic glutamate receptors are initially enhanced in ~1 min after starting the hypoxic episode, and then subsequently suppressed as the hypoxic episode continues. Synaptic responses recover fully if reoxygenation occurs in ~4 min. The early hypoxic enhancement likely results from the facilitation of glutamate release, via Ca2+ release from intracellular stores and subsequent increase in intracellular Ca2+ (Belousov et al. 1995; Katchman and Hershkovitz 1993a). The later suppression may be due to a failure in energy supply (Chung et al. 1998; Kass and Lipton 1989) and/or adenosine-dependent inhibition of glutamate release (Gribkoff and Bauman 1992; Hershkovitz et al. 1993; Katchman and Hershkovitz 1993b; Martin et al. 1994; Wu and Saggau 1994). Interestingly, synaptic responses mediated by N-methyl-d-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have been shown to be potentiated in slices after brief hypoxia (Gozlan et al. 1994; Hammond et al. 1994; Hsu and Huang 1997) or after temporary block of glycolysis (Teka Kok and Krnjević 1995, 1996). Because hypoxia or ischemia is known to cause elevation of intracellular Ca2+ (Choi 1995; Haddad and Jian 1993; Lobner and Lipton 1993; Meyer 1989; Mitani et al. 1994; Sieszjö and Bengtsson 1989; Silver and Erecinska 1990), a critical factor for synaptic plasticity (Bliss and Collingridge 1993), it is of interest to know whether alterations of glutamate transmission occur in intermediate times after brief hypoxia.

To test the above possibility, we conducted a brief hypoxic-hypoglycemic (HH) episode in adult rat brain slices, by exposing slices to a glucose-free medium aerated with 95% N2-5% CO2 rather than 5% O2-95% CO2, for ~4 min. Different from previous studies that monitor CA1 synaptic responses during and shortly (~2 h) after brief hypoxia, we made recordings in slices at the intermediate times post-HH (~8 h). To study the Ca2+-dependent glutamate release, CA1 synaptic potentials were evoked during perfusing slices with modified media that contained either the Ca2+ channel blocker Co2+, high Mg2+-low Ca2+, or the membrane permeant calcium chelator bis-(o-aminophenoxy)-N,N′,N′,N′-tetraacetic acid-AM (BAPTA-AM). We show that in slices recorded ~8 h postchallenge, CA1 synaptic responses were comparable in amplitude to those of controls but reacted differently from controls to the treatments designed to alter intracellular Ca2+ signals.

METHODS

Hypoxic challenge and electrophysiological recordings in brain slices have been previously described (Chung et al. 1998; Perez-Velaquez and Zhang 1994; Zhang and Krnjević 1993). Briefly, male Wistar rats (200–300 g) were deeply anesthetized with halothane and decapitated. To minimize the consequence of decapitation hypoxia, the brain was quickly dissected out (within 45 s) and maintained in an ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) for ~3–20 min before slicing. Hemisectioned brain was then mounted on an aluminum block, and cut transversely (400 μm thicknesses) using a vibrotom (series 1000, Tech. Prod. International, St. Louis, MO). The ACSF contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 glucose, with pH 7.4 when aerated with CO2 5%-O2 95% and 300 ± 5 mosM. For conducting a HH challenge, glucose in the ACSF was replaced equimolarly with sucrose, and 5% CO2-95% N2 rather than 5% CO2-95% O2 was used for aeration. After slicing, brain sections were kept in a glass container filled
with the warm (32–33°C), oxygenated (5% CO₂-95% O₂) ACSF for at least 1 h before further manipulations. After 1 h of stabilization, about half of the slices were gently transferred to another container filled with the glucose-free ACSF and aerated with 5% CO₂-95% N₂ (HH), for a period of 3.5–4 min. After the HH challenge, slices were returned to the original container and maintained there together with the control slices. To control effects due to mechanical disturbance associated with the HH challenge and for identification, each control slice was placed on a small piece of filter paper using a fine painting brush while kept in the original oxygenated container. By holding the filter paper, the slice was then taken out of the ACSF for a few seconds, and an incision was made cutting the thalamo–brain stem portion through the filter paper. After cutting, the slices were returned to the original container immediately, and the attached filter paper was removed. We found no evident change in CA1 electrophysiological responses recorded from the cut slices as compared with nontreated slices (Ouanonou et al. 1996; Zhang et al. 1994). Both control and challenged slices were maintained in the same container, and during the in vitro maintenance 20–30% of the medium in the container was replaced with the fresh ACSF every 2 h. The ACSF temperature in the container was maintained at 32–33°C using a water bath.

For electrophysiological recordings, slices were transferred to a fully submerged chamber and perfused with oxygenated (5% CO₂-95% O₂) ACSF continuously. Humidified, warm air of 5% CO₂-95% O₂ was also applied over the perfusate to increase oxygen tension in the local environment. All recordings were done at the bath temperatures of 32–33°C. Synaptic field potentials were recorded extracellularly using a glass pipette filled with 150 mM NaCl. The recording pipette was placed in the stratum pyramidale (soma) or stratum radiatum (dendrite) of the CA1 region, respectively. The Schaffer collateral pathway was stimulated electrically, by placing a bipolar tungsten electrode in the stratum radiatum at the CA1-CA2 border. Constant current pulses of 0.1 ms duration were generated through a Grass S88 stimulator and delivered through an isolation unit every 15–20 s. To examine paired pulse facilitation (PPF) in dendritic synaptic responses, twin stimuli at 50% of the maximal intensity were delivered at an interpulse interval of 50 ms. The PPF was determined by measuring the percent increase in the peak amplitude of the second response, taking the first response as 100%.

For whole cell recordings, we used a patch pipette solution containing (in mM)150 potassium methylsulfate, 2 HEPES, 0.5 ATP, and 10 EGTA, with pH 7.25 adjusted with KOH and 280 ± 10 mosM (Zhang et al. 1994). Filled with this solution, the tip resistance of the patch pipette was ∼4 MΩ. Electrical signals were recorded using an Axoclamp 2A amplifier or Axopatch amplifier 200 B (Axon Instruments, CA). The low-pass single-pole filter of the Axoclamp amplifier was set at 1 kHz, and the Bessel filter of the Axopatch amplifier (200B) was set at 2 or 5 kHz. After breaking through membrane, series resistance was usually <15 MΩ, and resistance compensation of the patch amplifier was set near 80%. Data were acquired, stored, and analyzed with Pclamp software (Version 5.5 or 6.3, Axon Instruments), through a 12-bit A/D interface (TL-1 or Digitata 1200, Axon Instruments).

To elicit minimal synaptic responses, Schaffer collateral afferent fibers were stimulated by a glass pipette filled with 150 mM NaCl. The location and intensity of afferent stimulation were adjusted until all-or-none excitatory postsynaptic currents (EPSCs) were observed. Evoked minimal EPSCs were sampled in the whole cell voltage-clamp mode at holding potentials more negative than −70 mV. Minimal EPSCs were included into data analysis if they fulfilled the following criteria: 1) EPSCs displayed small amplitudes and fast decay; 2) failure rate and amplitude distribution of EPSCs were independent of stimulation intensity in the range of approximately fivefold above the threshold stimulus; and 3) EPSCs were stable for at least 7–8 min when evoked at 0.1 Hz (cf. Allen and Stevens 1994; Raastad et al. 1992). Due to the limitation of the signal/noise ratio, failure of synaptic transmission was considered if the evoked responses were <5 pA and/or lack of a fast rising phase. The failure rate of EPSCs (%) for each individual neuron was calculated from >50 consecutive measurements.

To examine the amplitude distribution of minimal EPSCs, the EPSCs collected from a group of neurons were pooled together (n = 16 or 19 from control or challenged slices) and binned every 5 pA. The mean amplitude of each binned group was plotted versus the number of EPSCs included in the group (Fig. 6). The decay time course of the EPSC was determined by fitting a single exponential function to each individual event, using the Pclamp software (version 6.3, Axon Instruments).

All solutions were made with deionized sterile water (pH 5–6, resistance 18.2 MΩ/cm) from a Milli-Q UV plus system. Chemicals for making the patch pipette solutions were purchased from Fluka (New York, NY), except potassium methylsulfate (ICN, New York). BAPTA-AM was purchased from Molecular Probes (Eugene, OR). BAPTA-AM was dissolved initially in DMSO as a stock solution and then appropriately diluted to the ACSF. The final concentration of DMSO in the ACSF was ≤0.2%. To control the effect of DMSO, equal amount of DMSO was included in the control solution before the application of BAPTA-AM. Other drugs were purchased from Sigma (St. Louis, MO) or Tocris Cookson (Ballwin, MO). Means ± SE are given throughout the text.

**RESULTS**

**CA1 synaptic field potentials measured from control or HH challenged slices**

In control slices incubated in the ACSF at 32–33°C for ≤8 h, stimulation of the Schaffer collateral pathway induced synaptic field potentials in both somatic and dendritic areas of the CA1 neurons. In response to afferent stimulation at maximum intensity, the amplitudes of the somatic population spike and dendritic field excitatory postsynaptic potential (EPSP) were 4.5 ± 0.5 mV and 1.6 ± 0.5 mV, respectively (Table 1, Fig. 1A). When stimulated with the PPF paradigm (see METHODS), the dendritic field EPSP following the second stimulus was enhanced by 35.2 ± 3.2% (n = 7, Table 1, Fig. 1B). Perfusion of slices with 10 μM CNQX, a potent AMPA/kainate receptor antagonist, attenuated the dendritic field EPSPs by 83.4 ± 9.2% (n = 4), suggesting their mediation by non-NMDA glutamate receptors (Shimoo et al. 1997; Zhang et al. 1997).

We then recorded CA1 synaptic field potentials in slices at 6–8 h post-HH. In response to the maximal afferent stimulation, the amplitudes of CA1 synaptic responses measured post HH were not significantly different from those of control slices with 10 μM DMSO in the ACSF for 6–9 h after dissection. The hypoxic-hypoglycemic (HH) challenge was conducted at 1 h after slicing, by exposure of slices to a glucose-free ACSF aerated with 95% N₂-5% CO₂ rather than 95% O₂-5% CO₂, for ~4 min. After the HH episode, slices were maintained in the oxygenated normal ACSF for up to 8 h until recordings. *p < 0.05, control vs. post-HH, ANOVA (Bonferroni t-test). EPSPs, excitatory postsynaptic potentials; PPF, pair pulse facilitation.

**TABLE 1. Synaptic field potentials of CA1 neurons in control and post-HH challenged slices**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Post-HH (6–8 h)</th>
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<tr>
<td>Somatic population spikes, mV</td>
<td>4.5 ± 0.5 (12)</td>
<td>3.9 ± 0.7 (18)</td>
</tr>
<tr>
<td>Dendritic field EPSPs, mV</td>
<td>1.6 ± 0.2 (12)</td>
<td>1.3 ± 0.2 (13)</td>
</tr>
<tr>
<td>PPF in dendritic field EPSPs, %</td>
<td>35.2 ± 3.2 (7)</td>
<td>56.8 ± 10.1 (5)*</td>
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Values are means ± SE with number of neurons in parentheses. Control responses were collected from slices maintained in the artificial cerebrospinal fluid (ACSF) for 6–9 h after dissection. The hypoxic-hypoglycemic (HH) challenge was conducted at 1 h after slicing, by exposure of slices to a glucose-free ACSF aerated with 95% N₂-5% CO₂ rather than 95% O₂-5% CO₂, for ~4 min. After the HH episode, slices were maintained in the oxygenated normal ACSF for up to 8 h until recordings. *p < 0.05, control vs. post-HH, ANOVA (Bonferroni t-test). EPSPs, excitatory postsynaptic potentials; PPF, pair pulse facilitation.
Relation between CA1 synaptic responses and calcium influx after the HH challenge.

CA1 downward population spike in slice after the incubation in ACSF for similar suppression as controls following applications of 10 μM CoCl$_2$. Each concentration of CoCl$_2$ was applied ACSF that contained 0.01, 0.1, 0.3, 0.5, 1, or 2 mM CoCl$_2$. In slices recorded 3–6 h after the HH challenge (n = 9), CA1 field EPSPs were comparable with those in the control group, with a mean amplitude of 1.9 ± 0.2 mV measured in the standard ACSF. However, significant decrease in the field EPSPs was not observed until external CaCl$_2$ reached the concentration of ≥0.5 mM. The dose-response plot calculated for the challenged slices showed a rightward shift as compared with the control, with an EC$_{50}$ of 1.2 mM for external CoCl$_2$ (Fig. 2, A1 and A2). Neither control nor challenged slices showed proportional decreases in presynaptic volleys following applications of CoCl$_2$ (Fig. 2, A1 and A2), suggesting that decreases in synaptic potentials by CoCl$_2$ result largely from reduction in Ca$^{2+}$-dependent glutamate release rather than attenuation of local excitability of afferent axon fibers.

To examine effects of external Ca$^{2+}$ on the CA1 synaptic potentials and to control possible nonspecific effects of CoCl$_2$, we modified the ACSF by keeping external Mg$^{2+}$ constant at 4 mM and reducing external Ca$^{2+}$ from 2 mM to 1, 0.5, or 0.1 mM, respectively. The aim of this approach was to reduce transmembrane Ca$^{2+}$ gradient and hence the Ca$^{2+}$ influx associated with the afferent impulse. Slices were exposed sequentially to the low-Ca$^{2+}$ ACSF for ≥8 min to ensure the steady-state response. In control slices incubated 2–7 h after dissection, CA1 field EPSPs evoked by the maximal afferent stimulation had amplitudes of 1.7 ± 0.2 mV in the presence of 2 mM external Ca$^{2+}$. Lowering external Ca$^{2+}$ from 2 mM to 1, 0.5, or 0.1 mM decreased CA1 field EPSPs by 21.7 ± 5.0%, 58.5 ± 6.0%, or 90.2 ± 1.8%, respectively (n = 10, Fig. 2, B1 and B3).

In slices examined 2–6 h post-HH challenge, amplitudes of CA1 field EPSPs were comparable with the controls, with a mean value of 1.5 ± 0.1 mV (n = 12) in the presence of 2 mM external Ca$^{2+}$. However, lowering external Ca$^{2+}$ caused smaller changes in the post-HH EPSPs than controls, and the mean decreases were 6.4 ± 5.3%, 33.4 ± 11.2%, or 62.9 ± 6.5% as external Ca$^{2+}$ was reduced to 1, 0.5, or 0.1 mM, respectively (Fig. 2, B2 and B3). Neither control nor challenged slices showed attenuation in presynaptic volleys when perfused with low-Ca$^{2+}$ medium (Fig. 2, B1 and B2). These observations, when taken together with the differential effects of CoCl$_2$ mentioned above, suggest an alteration in Ca$^{2+}$-dependent glutamate transmission in slices following a HH challenge.

Failure of BAPTA-AM to attenuate CA1 synaptic potentials in slices post-HH

We then examined the effects of BAPTA-AM, a membrane-permeant calcium chelator, on CA1 dendritic field EPSPs, in an attempt to buffer intracellular Ca$^{2+}$ associated with the afferent impulse. To promote intracellular accumulation of BAPTA, 0.5–1 mM probenecid, an anion transporter inhibitor, was included in the perfusate before and during the applications of BAPTA-AM (Ouanonou et al. 1996, 1999). At the concentrations we used, probenecid caused no consistent change in the CA1 field EPSPs, change in the field EPSPs was stabilized. In control slices following 3–6 h of in vitro incubation, CA1 field EPSPs of 1.8 ± 0.1 mV (n = 8) were observed before CoCl$_2$ application. Following applications of CoCl$_2$, these EPSPs showed a concentration-dependent decrease in amplitude, with a significant decrease starting from 0.3 mM CoCl$_2$ and a calculated EC$_{50}$ of 0.5 mM for external CoCl$_2$ (Fig. 2, A1 and A3).
but transient decreases in field EPSPs were observed in some slices with unknown mechanisms. To control side effects of this agent, slices were perfused with 0.5–1 mM probenecid for 15 min until stable field EPSPs were achieved, and the same concentration of probenecid was present in the BAPTA-containing perfusate throughout the application period.

In control slices incubated 4–8 h in vitro, the mean amplitude of CA1 dendritic field EPSPs was 0.9 ± 0.1 mV (n = 16) following the afferent stimulation near 50% of the maximum strength. After applications of BAPTA-AM (10 μM, 20–25 min), these field EPSPs were decreased by 43.6 ± 9.4% (P < 0.001, paired t-test; Fig. 3, A and C), as measured at the end of BAPTA-AM application. The decreases in field EPSPs were partially reversible after washing BAPTA-AM for 30 min, suggesting a suppression of synaptic potentials by the calcium chelator rather than a time-dependent deterioration in synaptic responses.

In slices (n = 11) that had recovered for 4–8 h after the HH challenge, CA1 field EPSPs were comparable in amplitude to controls, with a mean value of 0.8 ± 0.1 mV evoked by the half-maximal stimulation. However, in contrast to the control response, applications of 10 μM BAPTA-AM caused no decrease, but rather lead to a small increase by 16.1 ± 4.7% (P = 0.05, paired t-test) in the field EPSPs (Fig. 3, B and C). These changes induced by BAPTA-AM were significantly different from those in controls (P < 0.01, nonpaired t-test). Small increases in CA1 field EPSPs by BAPTA-AM were also observed at other post-HH times, with a mean change of 19.8 ± 10.1% (n = 7) or 21.3 ± 7.3% (n = 6) in slices recovered 1–3 and 9–12 h after the HH challenge, respectively.

**Minimal EPSCs in CA1 neurons post-HH**

The above data were obtained by using extracellular recordings of synaptic field potentials, which represent summed responses from a population of glutamate synapses.
To examine the post-HH changes in separate glutamate synapses, CA1 pyramidal neurons were recorded in the whole cell voltage-clamp mode, and EPSCs were evoked using a minimal stimulation paradigm (Allen and Stevens 1994; Raastad et al. 1992) (see also METHODS). To block the voltage-dependent and Mg$^{2+}$-sensitive synaptic responses mediated by NMDA receptors, CA1 neurons were held at potentials of −70 to −80 mV. Bicuculline methiodide (10 μM) was bath applied throughout the recording period to block synaptic currents mediated by GABA_A receptors. Once the whole cell recordings were achieved, the stimulation intensity and position were adjusted until small, all-or-none responses were obtained, such that the amplitude and failure rate of the evoked EPSCs were independent from the stimulation intensity in a certain range (see METHODS). Two identical stimuli separate by 30 ms were delivered every 30 s. It is expected that the second EPSC would have kinetics similar to that of the first one if they originate from the same, but limited release sites. Control neurons (n = 16) were recorded from slices maintained in vitro for 2–8 h after sectioning, and challenged neurons (n = 19) were recorded from slices 2–6 h after the HH challenge.

In control CA1 neurons (n = 16), minimal EPSCs displayed a large variability in their amplitude, ranging from 5 to 120 pA. To reveal the amplitude distribution of the minimal EPSCs, synaptic currents evoked by the first or second stimulus were pooled together and binned every 5 pA. Then the mean amplitudes were plotted versus the number of events included in each bin (Fig. 4A). Of the 576 events evoked after the first stimulus, EPSCs with amplitude of ≥55 pA accounted for 28.3% of the total events (Fig. 6A). In response to ≥50 consecutive afferent stimuli in each neuron examined, minimal EPSCs exhibited mean failure rates of 69.9 ± 2.1% and 48.6 ± 3.3% (Fig. 4, B and C) as evoked by the twin stimuli. We measured the rise time and the decay time constant of EPSCs. The latter was determined by computing a single exponential fit to each individual event. Following the twin minimal stimuli, EPSCs displayed a mean amplitude of 40.8 ± 2.1 pA, rise time of 2.8 ± 0.1 ms, and decay time constant of 7.2 ± 0.2 ms, as measured from 576 and 796 events, respectively. The comparable kinetics between the twin EPSCs implies that a small number of synapses are activated after the minimal stimulation. When the amplitudes of EPSCs evoked by the first stimulus were plotted versus their rise time or decay time constant, no significant correlation was found between these parameters (linear correlation coefficient factor $R^2 < 0.1$). These observations are consistent with previous studies (Allen and Stevens 1994; Raastad et al. 1992), suggesting that the measurement errors owing to the space-clamp limitation is not the dominant factor responsible for the variability in the EPSC amplitude observed.

Minimal EPSCs were readily evoked from CA1 neurons (n = 19) in slices post-HH. The amplitude distribution of these EPSCs was slightly different from that observed in controls, such that a total of 416 events evoked by the first stimulus, only 41 EPSCs (10%) had amplitudes of ≥55 pA (Fig. 4D). The failure rate of first or second EPSCs was
59.4 ± 1.9% or 37.5 ± 2.6%, which is slightly but significantly lower than that in controls (69.9 ± 2.1% or 48.6 ± 3.3%, P < 0.05, Student’s t-test). In addition, spontaneous EPSCs or delayed EPSCs following afferent stimulation (Fig. 4E) were often observed in CA1 neurons post-HH challenge. As found in control neurons, EPSCs evoked by the first and second stimulus shared similar kinetic parameters, with amplitudes of 29.1 ± 0.9 and 32.1 ± 0.9 pA, rise times of 3.6 ± 0.1 and 3.3 ± 0.1 ms, and decay time constants of 9.8 ± 0.4 and 8.1 ± 0.5 ms (n = 381 and 416), respectively. No significant correlation was found between the amplitude and rise time or decay time constant of these EPSCs (linear regression coefficient factor $R^2 < 0.1$). However, the decay time constants of first EPSCs were significantly longer in post-HH neurons than controls (P < 0.05), which may reflect changes in postsynaptic AMPA receptors as suggested in posts ischemic neurons (Gorter et al. 1997; Pellegrini-Giampietro et al. 1993; Rump et al. 1996; Tsubokawa et al. 1994; urban et al. 1989).

**DISCUSSION**

In the present experiments, brief HH was made by exposure of adult rat brain slices to glucose-free ACSF aerated with nitrogen rather than oxygen for ~4 min. We chose this in vitro HH model because the challenge shares some common features with ischemic insults in vivo, particularly an over-stimulation of glutamate NMDA receptors and disruption of normal Ca$^{2+}$ homeostasis (Kass and Lipton 1986; Michaels and Rothman 1990). In previous studies the similar HH paradigm has been shown to cause a reversible depression in CA1 synaptic potentials (Hammond et al. 1994; Perez-Velazquez and Zhang 1994; Small et al. 1997), an elevation of intracellular Ca$^{2+}$ homeostasis (Kass and Lipton 1986; Michaels and Rothman 1990), and alterations in gene expression in hippocampal slices postchallenge (Charriaut-Marlangue et al. 1992; Perez-Velazquez and Zhang 1994). Extracellular recordings of dendritic field potentials were used in most of the present experiments because these responses represent the summed activity resulting from a population of glutamate.
synapses and they are much more stable as compared with single-cell recordings during experimental manipulations. We present convergent evidence suggesting that a brief HH challenge may cause significant alterations in the Ca\(^{2+}\) dependence of glutamate transmission in hippocampal CA1 region.

In an attempt to attenuate the Ca\(^{2+}\) influx associated with the afferent impulse, slices were perfused with a modified ACSF containing Co\(^{2+}\) or high Mg\(^{2+}\)-low Ca\(^{2+}\). We found that these two treatments attenuated CA1 synaptic potentials in both control and post-HH slices, but the extent of attenuation was smaller in the challenged slices than in controls. The EC\(_{50}\) for external Co\(^{2+}\) to attenuate CA1 synaptic potentials was increased from 0.5 mM in control to 1.2 mM in challenged slices (Fig. 2A). Similarly, lowering external Ca\(^{2+}\) from 2 to 0.1 mM decreased CA1 synaptic response by 90% in control, but only 60% in slices postchallenge (Fig. 2B). Neither applying Co\(^{2+}\) nor lowering external Ca\(^{2+}\) changed the presynaptic volley in both groups of slices, suggesting that the differences observed between these two groups are largely due to alterations in the Ca\(^{2+}\)-dependent glutamate release. However, possible alterations of postsynaptic glutamate receptors, as observed in postischemic neurons (Gorter et al. 1997; Pellegrini-Giampietro et al. 1993; Rump et al. 1996; Tsubokawa et al. 1994; Urban et al. 1989), and their responses to the Co\(^{2+}\) treatment may also be factors yet to be determined.

BAPTA-AM was used as an alternative to attenuate intracellular calcium signals without blocking calcium channels or reducing transmembrane Ca\(^{2+}\) gradient. Previous studies have shown attenuated synaptic transmission by BAPTA-AM in mammalian CNS neurons and other neural preparations, largely due to buffering intracellular Ca\(^{2+}\) responsible for the transmitter release processes (Adler et al. 1991; Niesen et al. 1991; Ouanoou et al. 1996, 1999; Spigelman et al. 1996; Tymianski et al. 1994). We show here that external BAPTA-AM is effective in decreasing the CA1 field EPSPs in control slices, in keeping with previous studies mentioned above, but the similar BAPTA-AM application was ineffective to decrease CA1 field EPSPs in slices post-HH. The later observation is consistent with resistance of the challenged CA1 synaptic potentials to the Co\(^{2+}\) or low Ca\(^{2+}\) treatment. Collectively, these observations suggest altered dynamics between intracellular Ca\(^{2+}\) and release processes in CA1 glutamate synapses post-HH, by which these synapses become insensitive to a moderate decrease in intracellular Ca\(^{2+}\) at levels sufficient to attenuate glutamate release in controls. It remains to be clarified whether BAPTA loading or distribution in challenged presynaptic terminals is comparable with that in control. A direct measurement of Ca\(^{2+}\) signals in presynaptic terminals may provide some clues regarding this issue (Wu and Saggau 1993, 1994).

To demonstrate the hypoxic alterations occurring at individual CA1 glutamate synapses, we monitored glutamate EPSCs from singly recorded CA1 pyramidal neurons using a minimal stimulation paradigm (Allen and Stevens 1994; Raastad et al. 1992). The minimal EPSCs displayed an all-or-none like response, presumably reflecting glutamate release following the impulses generated from a few afferent fibers. If the processes responsible for the Ca\(^{2+}\)-dependent glutamate release are promoted after the HH challenge such that intracellular Ca\(^{2+}\) signals are more efficient than normal to induce functional transmission, one may expect a lower EPSC failure rate in challenged neurons. In keeping with this view, minimal EPSCs were readily observed in recordings from post-HH neurons, with the failure rate slightly but significantly lower than that of controls.

However, the data regarding minimal EPSCs must be interpreted with caution. First, because of the limitations in the signal/noise ratio and the effectiveness of the space clamp, particularly for synapses electrotonically distant from the somatic recording, the amplitude and/or kinetics of EPSCs may not be accurately measured. EPSCs with small amplitudes may not be detected under our recording conditions, therefore causing measurement errors in assessing the failure of minimal EPSCs. Second, the failure rate of minimal EPSCs represents not only the probability in transmitter release, but also the functional state of axon conduction and/or intrinsic axonal excitability (Allen and Stevens 1994). In extracellular recordings, CA1 presynaptic volleys with large amplitude seem to be frequently observed in slices post-HH but with large variability. No intention was made in the present study to compare the amplitude/waveform of CA1 presynaptic volleys between control and challenged slices, because of difficulty in controlling the precise transverse slicing plan hence the preservation of Schaffer collateral afferent fibers in each individual slice. However, the issue remains as to whether there are HH-induced changes in the intrinsic excitability of Schaffer collateral fibers. Third, previous studies have revealed a wide heterogeneity in morphology and functionality of CA1 glutamate synapses (see review by Edwards 1995). For example, at least two classes of glutamate synapses with a sixfold difference in their release probability have been noted in the Schaffer collateral-CA1 pathway. The synapses with lower release probability contribute over half of the transmission examined in the standard slice recording conditions (Hessler et al. 1993). Also, glutamate synapses may undergo activity-dependent changes in release probability (Goda and Steven 1994) and alterations in postsynaptic AMPA/kainate receptors following the ischemic insult (Benke et al. 1998; Gorter et al. 1997; Hu et al. 1998; Pellegrini-Giampietro et al. 1993; Rump et al. 1996; Tsubokawa et al. 1994; Urban et al. 1989). Thus it may well be that synapses with altered release probability and/or postsynaptic receptors are involved in our samplings in control and/or post-HH slices. In viewing the above complications, it is tentatively suggested that there may be an increase in the likelihood of transmitter release at CA1 glutamate synapses post-HH challenge.

We propose that the brief HH challenge causes alterations in Ca\(^{2+}\)-dependent processes that govern the evoked transmitter (glutamate) release, such that these processes become more sensitive to the intracellular Ca\(^{2+}\) signals associated with the afferent impulse. Thus a moderate reduction of intracellular Ca\(^{2+}\) signals, by buffering intracellular Ca\(^{2+}\), blocking voltage-gated Ca\(^{2+}\) channels or lowering the transmembrane Ca\(^{2+}\) gradient, would have weaker influence on glutamate synaptic potentials in challenged slices than controls. Several factors may be responsible for the altered synaptic physiology, including 1) elevated intracellular Ca\(^{2+}\) owing to enhanced entry, retarded buffering, and/or removal, 2) increased sensitivity of synaptic proteins or release processes to corresponding intracellular Ca\(^{2+}\) signals, 3) involvement of more synapses with higher release probability. We have no direct evidence supporting these possibilities at present. Considering the dynamic
interaction between the intracellular Ca$^{2+}$ and synaptic proteins (Sheng et al. 1996) and the resulting differential modulations on transmitter release (Mochida et al. 1996), it is conceivable that the multimechanisms may be involved in the post-HH alterations in CA1 glutamate transmission. Potentiation of NMDA or AMPA receptor–mediated EPSPs has been found in the CA1 region of slices after brief hypoxia or temporary inhibition of glycolysis with 2-deoxyglucose, termed “anoxic long-term potentiation” (anoxic LTP) (Hammond et al. 1994; Hsu and Huang 1997) or “2-DG-LTP” (Tekökk and Krnjević 1995). Complexes of cellular mechanisms are involved in inducing the anoxic LTP or 2-DG-LTP, including activation of NMDA receptors, Ca$^{2+}$ entry and subsequent increase in intracellular Ca$^{2+}$, and nitric oxide production (Crepel and Ben-Ari 1996; Huang and Hsu 1997; Tekökk and Krnjević 1996). Although we have not examined the nature of NMDA receptor activation and the rise of intracellular Ca$^{2+}$ involved in the HH episode we employed, it is likely that similar signal transduction cascades may also account for the apparent facilitation of CA1 glutamate transmission post-HH. Our data support the previous studies mentioned above, further suggesting that the apparent alteration of CA1 glutamate transmission can persist for several hours after brief HH.

In summary, the present experiments show that brief HH causes a substantial alteration in CA1 glutamate potentials that last several hours postchallenge. This is manifested by the findings that challenged CA1 synaptic potentials are less sensitive to procedures reducing intracellular Ca$^{2+}$, and unitary glutamate EPSCs are more persistent as compared with controls. We propose that the evoked glutamate transmission is facilitated post-HH, as the result of alteration of Ca$^{2+}$-dependent processes that govern the release processes. It remains to be shown whether similar alterations take place in CA1 glutamate synapses after transient ischemia in vivo, and if so, how they are related to the delayed CA1 neuronal degeneration (Kirino 1982; Pulsinelli et al. 1982; Shinno et al. 1997, Zhang et al. 1997).

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REFERENCES


Kass, I. S. and Lipton, P. Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the hippocampal slices. J. Physiol. (Lond.) 378: 313–334, 1986.

Kass, I. S. and Lipton, P. Protection of hippocampal slices from young rats against anoxic transmission damage is due to better maintenance of ATP. J. Neurophysiol. 413: 1–11, 1989.


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