Mechanisms Underlying the Depression of Evoked Fast EPSCs Following In Vitro Ischemia in Rat Hippocampal CA1 Neurons

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INTRODUCTION

In rat hippocampal slice preparations, oxygen and glucose deprivation (in vitro ischemia) causes a stereotyped response in CA1 neurons, which is characterized by an initial hyperpolarization, and a subsequent slow depolarization followed by a rapid depolarization after approximately 6 min of ischemic exposure. When the rapid depolarization occurs, the membrane potential can thereafter never again be restored, even if oxygen and glucose are reintroduced (Tanaka et al. 1997). In addition, the fast excitatory postsynaptic potentials (EPSPs) evoked by the focal stimulation of the Schaffer collateral show a decreased amplitude after a short period (4 min) of in vitro ischemia (Higashi 1995). In vitro ischemia increases the extracellular adenosine concentration in rat hippocampal slices, which is probably due to a breakdown of the intracellular ATP (Pedata et al. 1993). Adenosine inhibits evoked EPSPs in the hippocampal CA1 neurons (Siggins and Schubert 1981). In cultured hippocampal neurons, the evoked excitatory postsynaptic currents (EPSCs) but not inhibitory postsynaptic currents (IPSCs) were also depressed by adenosine (Yoon and Rothman 1991). Moreover, adenosine decreases the K+−stimulated glutamate (Glu) release and inhibits N-type Ca2+ channels of both the CA3 pyramidal cell body (Mogul et al. 1993) and the excitatory presynaptic terminal projected to CA1 neurons (Wu and Saggau 1994). Adenosine receptor antagonist, 8-cyclopentyltheophylline (8-CPT), attenuates the hypoxia-induced depression of the evoked population spikes and postsynaptic field potentials in hippocampal slices (Fowler 1990; Gribkoff et al. 1990). These findings suggest that the depression of evoked EPSPs by in vitro ischemia probably results from the depression of Glu release from the presynaptic axon terminal, which may be mediated by presynaptic adenosine receptors.

On the other hand, low concentrations (10 nM to 1 μM) of adenosine augment the evoked EPSPs in hippocampal CA3 neurons (Sakurai and Okada 1992). In addition, hypoxia-induced hyperpolarization is mainly mediated by ATP-sensitive K+ channels (Fujimura et al. 1997). Exogenous adenosine produces a hyperpolarization associated with a decrease in the membrane resistance (Okada and Ozawa 1980; Segal 1982) and increases K+ conductance (Gerber et al. 1989) in the rodent CA1 neurons. It is therefore possible that the depression of the evoked EPSPs and the postsynaptic field potentials produced by in vitro ischemia may be due to a shunting effect of the neuronal membrane of the CA1 neurons. The mechanisms, therefore underlying the depression of the evoked EPSP or EPSC by in vitro ischemia have yet to be clarified.

The present study is concerned with the mechanisms involved in the depression of evoked fast EPSCs induced by in...
vitro ischemia in hippocampal CA1 neurons in the slice preparation of adult rat. We have examined whether the depression of the fast EPSCs is mediated via presynaptic adenosine 1 (A1) receptors, and if so, how much does the activation of presynaptic A1 receptor contribute to the depression. Furthermore, we have studied other factors that may induce the depression of the evoked fast EPSCs during in vitro ischemia. The present results suggest that the evoked fast EPSCs were markedly depressed by presynaptic A1 receptors during in vitro ischemia. In the presence of a maximal concentration (1–10 μM) of a selective A1 receptor antagonist, 8-CPT, however, the evoked fast EPSCs were still depressed by in vitro ischemia. The remaining depression by in vitro ischemia in the presence of 8-CPT is probably due to a direct inhibition of the Ca2+ influx to the axon terminals. Preliminary accounts of some of the above data have already been presented in abstract form (Tanaka et al. 1999).

METH ODS

All experiments were conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan and had the approval of the Institutional Animal Use and Care Committee in Kurume University. Wistar rats (male, 250–350 g; 8–12 wk old) were rapidly decapitated under ether anesthesia, and the forebrains were removed and placed in chilled (4–6°C) Krebs solution that was aerated with 95% O2–5% CO2. The composition of Krebs solution was (in mM) 117 NaCl, 3.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 25 NaHCO3, and 11 glucose. The hippocampus was dissected and then sliced (thickness of 400 μm) with a Vibratome (Oxford). A slice was placed on a nylon net in a recording chamber (volume, 500 μl) and immobilized with a titanium grid placed on the upper surface of the section. The preparation was completely submerged in the superfusing solution (temperature at 36.0 ± 0.5°C, flow at 4–6 ml/min).

Intracellular recordings were made from CA1 and CA3 pyramidal neurons with 2 M K acetate–containing electrodes (resistances of 50–70 MΩ). Extracellular DC potential recordings in proximal stratum radiatum (200–300 μm apart from the stratum pyramidale) of the CA1 region were obtained with Krebs solution– containing electrodes (resistances of 30–50 MΩ). In the conventional intracellular recording, the apparent input resistance in CA1 neurons was monitored by passing small hyperpolarizing pulses (0.1–0.2 nA, 200 ms) through the recording electrode. In the presence of 8-CPT (1 μM), nifedipine (20 μM), tetrodotoxin (TTX, 0.5 μM), and tetroethylammonium chloride (TEA, 20 mM), the nifedipine-resistant, Ca2+–dependent action potential was evoked by passing depolarizing pulses (1.3–2.8 nA, 20 ms) in CA3 neurons, and the amplitude was measured from the resting membrane potential to the peak potential.

To record evoked fast EPSCs, the electrodes filled with 2 M K acetate were also used for single-electrode voltage-clamp experiments. Voltage-clamp recordings were obtained with a single-electrode voltage-clamp amplifier (Axon Instruments, Axoclamp 2B), employing a switching frequency of 5 kHz and a 30% duty cycle. The cells were voltage-clamped at the membrane potential of ~80 mV. The headstage voltage was continuously monitored to ensure the absence of the pipette. In the presence of 8-CPT (1 μM), spontaneous miniature EPSCs were recorded and analyzed off-line using the Mini Analysis data analysis program (Synaptosoft). The events were semi-automatically detected in epochs measuring 30 s in duration using an adjustable amplitude threshold (typically set at ~3.5 pA) and then were held constant for a given experiment. The synaptic events were selected in a blinded fashion, and spurious events were manually rejected before the final calculation of the amplitudes. Baseline noise was determined from automatic measurements taken before each miniature EPSC. The baseline noise was measured 7 ms before each miniature EPSC.

Slices were made “ischemic” by medium equilibrated with 95% N2–5% CO2 and deprived of glucose, which was replaced with NaCl isosmotically (ischemia-simulating medium). When switching the superfusing media, there was a delay of 15–20 s before the new medium reached the chamber, due to the volume of the connecting tubing. As a result, the chamber was filled with the test solution approximately 30 s after switching the solution.

The drugs used were (+)-bicuculline (from Sigma), 8-cyclopentyltheophylline (8-CPT), nifedipine (all from RBI), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), dl-2-amino-5-phosphonopentanoic acid (AP5, all from Tocris), TTX (from Wako), and TEA (from Tokyo Kasei Organic Chemical).

For single-electrode voltage-clamp recordings, the membrane currents were filtered at 3 kHz and digitized 30 kHz. For both the conventional intracellular recording and the extracellular recording, the membrane potentials and the DC potentials were digitized at 30 and 50 kHz, respectively. The signals were digitally recorded by using Clampex 6 (Axon Instruments). The recorded signals were analyzed by using either Clampfit 6 or AxoGraph 3 (Axon Instruments). All quantitative results were expressed as the means ± SD. The number of slices examined is given in parentheses. The one-way ANOVA with the Scheffé post hoc test was used to compare the data. In some cases, either the Kolmogorov–Smirnov test or two-way ANOVA was used. Statistical significance was determined at the level of P < 0.05 unless otherwise indicated.

RESULTS

This study was mainly based on intracellular recordings from 77 CA1 pyramidal neurons of adult rats with stable membrane potentials more negative than ~60 mV. The resting membrane potential and the apparent input resistance in CA1 neurons were ~71 ± 5 mV (mean ± SD, n = 65) and 38 ± 10 MΩ (n = 65), respectively.

In vitro ischemia depressed the evoked EPSCs in CA1 neurons

The evoked fast EPSC was recorded from CA1 neurons by using single-electrode voltage-clamp method in the presence of bicuculline (10 μM). Unless stated otherwise, the control and ischemia-simulating media contained 10 μM bicuculline to eliminate inhibitory synaptic currents. The membrane potential was held at ~80 mV, and the fast EPSC was elicited by focal
stimulation of Schaffer collateral every 5–10 s. The fast EPSCs showed monosynaptic characteristics; i.e., they were constant in latency and configuration, and there were no failures during a train of 20 stimuli at 20 Hz. The amplitude of fast EPSC was $770 \pm 320 \text{ pA} (n = 20)$ at $4 \text{ V}$ of stimulus intensity, which was 1.5 times higher than the threshold intensity, in the control medium. The rise time of fast EPSC was $2.6 \pm 0.86 \text{ ms} (n = 20)$, and the decay time constant was $7.6 \pm 3.1 \text{ ms} (n = 20)$ in the control medium.

We examined the effects of superfusion with the ischemia-simulating medium for a short period (4 min) on the fast EPSC, since the prolonged application (more than 4 min) often induced a marked rapid inward current. Figure 1, A and B, shows typical changes in the fast EPSCs and in the time course of the depression, respectively, during ischemic exposure. The amplitude of the fast EPSC was reversibly suppressed by in vitro ischemia. The amplitude of fast EPSC was $238 \pm 186 \text{ pA} (n = 20, P < 0.01)$ 2 min after the onset of the ischemic exposure, and was $118 \pm 60 \text{ pA} (n = 20, P < 0.01)$ 4 min after onset. The mean amplitude of the fast EPSCs was reduced by $85 \pm 7\%$ of the control 4 min after the onset of ischemic exposure. In contrast, the mean rise time and decay time constant of the fast EPSCs were not significantly changed by the ischemic exposure.

To elucidate whether or not there is an inhibition of postsynaptic glutamate (Glu) receptors by ischemic exposure, exogenous Glu (3 mM) was superfused for 20 s. Figure 1C shows a typical Glu-induced inward current before ischemic exposure for 3 min and 5 min after washing out ischemic solution. The mean amplitude and duration of the Glu-induced inward currents ($367 \pm 84 \text{ pA}$ and $1.2 \pm 0.16 \text{ min}$, respectively, $n = 6, P < 0.01$) 3 min after the onset of ischemic exposure significantly increased in comparison to those ($230 \pm 77 \text{ pA}$ and $0.9 \pm 0.2 \text{ min}$, respectively, $n = 6$) in the control medium. The results suggest that postsynaptic Glu responses are not reduced during in vitro ischemia.

In vitro ischemia did not change the amplitude of spontaneous miniature EPSCs in CA1 neurons

To elucidate the mechanisms underlying the depression of the evoked fast EPSC by ischemic exposure, spontaneous miniature EPSCs were recorded in the presence of TTX (1 \(\mu\text{M}\)) and bicuculline (20 \(\mu\text{M}\)) using the whole cell patch-clamp method. The miniature EPSCs were reversibly abolished by 10-min application of CNQX (5 \(\mu\text{M}\); $n = 5$, not shown). Figure 2 shows the typical effects of ischemic exposure on the amplitude and frequency of the miniature EPSCs. The distribution of the amplitudes was skewed before, during, and after ischemic exposure as shown in Fig. 2B. However, the mode of the distribution did not change during ischemic exposure. Figure 2C shows the normalized cumulative distribution for the
amplitude. The cumulative distribution curve did not significantly change by ischemic exposure (Kolmogorov-Smirnov test). Figure 2D shows the normalized cumulative distribution for the inter-event interval. Compared to the control cumulative curve, ischemic exposure for 2 min significantly shifted the curve to the left (0.20 by Kolmogorov-Smirnov test), and the exposure for 4 min shifted it to the right (0.20 by Kolmogorov-Smirnov test). Similar shifts by 2 and 4 min ischemic exposure were obtained in all six neurons tested. As a result, the mean inter-event interval (301 ± 6120 ms, n = 6, P = 0.01) 2 min after the onset of in vitro ischemia was significantly longer than that (196 ± 53 ms, n = 6) in the control medium.

On the other hand, the mean inter-event interval (120 ± 47 ms, n = 6, P = 0.01) 4 min after the onset of in vitro ischemia was significantly shorter than that in the control medium. No change in the amplitude of miniature EPSCs suggests that the depression of the evoked EPSCs by in vitro ischemia is due to presynaptic mechanisms.

**In vitro ischemia-induced depression of evoked fast EPSC was antagonized by A1 receptor antagonist in CA1 neurons**

To elucidate the presynaptic inhibitory mechanism for the depression of the evoked fast EPSC, the effects of an antagonist for A1 receptors, 8-CPT, on the fast EPSCs before and during ischemic exposure were examined. When the hippocampal slices were superfused with 8-CPT (1 μM) for 15 min in the control medium, the fast EPSCs increased by 27 ± 9% (n = 8) of the control amplitude. The rise time and decay time constant of the fast EPSCs did not change. Figure 3 shows the typical effects of 8-CPT on the depression of the fast EPSCs during ischemic exposure. In the absence of 8-CPT, the fast EPSCs decreased in amplitude by 84 ± 9% (n = 8, P < 0.01) of the control 4 min after the onset of ischemic exposure (Fig. 3A, top traces, and B, ○). In contrast, the fast EPSCs in the presence of 8-CPT (1 μM) slightly decreased by 22 ± 13% (n = 8, P < 0.01 compared with the degree of inhibition in the absence of 8-CPT; Fig. 3A, middle traces, and B, ○). Figure 3C shows that the inhibition of the fast EPSCs following ischemic exposure was antagonized by 8-CPT, in a concentration-dependent manner; in the presence of 0.3 and 10 μM 8-CPT, the amplitude of the fast EPSCs decreased by 60 ± 10% (n = 6, P < 0.01) and 20 ± 10% (n = 6, P < 0.01) in comparison to the respective controls.

**In vitro ischemia depressed Ca2+ spikes of CA3 neurons in the presence of 8-CPT**

As mentioned above, 8-CPT at high concentrations (1, 10 μM) could not completely antagonize the in vitro ischemia-induced depression of the evoked fast EPSC. To access the possibility that the remaining reduction caused by ischemic...
exposure may be due to a decrease in the Ca\(^{2+}\) influx at the axon terminal, Ca\(^{2+}\)-dependent spikes were recorded from the CA3 neurons in the presence of 8-CPT (1 \(\mu\)M), TTX (0.5 \(\mu\)M), and TEA (20 mM). Figure 4A shows typical Ca\(^{2+}\)-dependent spikes; an initial spike was followed by a burst of spikes on a slowly depolarizing potential. Co\(^{2+}\) (2 mM) abolished all the responses in all neurons tested (\(n = 4\), not shown). Nifedipine (20 \(\mu\)M) blocked the burst of spikes and shortened the slowly depolarizing potential, but not the initial spike. Nifedipine slightly prolonged the half-width of the initial spike without affecting the amplitude, threshold, and maximal slope of the rising phase (dV/dr; Fig. 4A). We therefore examined the effects of ischemic exposure on the nifedipine-resistant Ca\(^{2+}\) spikes in 8-CPT (1 \(\mu\)M), TTX (0.5 \(\mu\)M), TEA (20 mM), and nifedipine (20 \(\mu\)M) containing medium (Fig. 4B). The amplitude and dV/dr of the nifedipine-resistant Ca\(^{2+}\) spike significantly decreased by 10 ± 14\% (\(n = 6\), \(P < 0.05\)) and 12 ± 6\% (\(n = 6\), \(P < 0.01\)) of the respective controls. The spike threshold significantly depolarized from -34 ± 8 mV of the control to -28 ± 3 mV (\(n = 6\), \(P < 0.05\)) 4 min after the onset of ischemic exposure. The half-width of the spike significantly decreased by 22 ± 28\% (\(n = 6\), \(P < 0.01\)) in comparison to the control. These results suggest that the nifedipine-resistant, Ca\(^{2+}\)-dependent currents may be directly depressed by in vitro ischemia (see DISCUSSION).

**In vitro ischemia enhanced paired-pulse facilitation**

When the Schaffer collaterals are stimulated twice in rapid succession, there is a characteristic facilitation of the field PSP elicited by the second impulse in CA1 region (Creager et al. 1980). The paired-pulse facilitation (PPF) of evoked EPSPs and EPSCs in hippocampal neurons has been found to be inversely correlated with the release probability of Glu (Dobrunz and Stevens 1997; Manabe et al. 1993; Zucker 1989). The relationship of the PPF ratio (the 2nd EPSC amplitude/the 1st EPSC amplitude \(\times 100\)) with the interstimulus interval and with the stimulus intensity was examined in normal Krebs solution using the single-electrode voltage-clamp technique. The PPF was observed at stimulus intervals shorter than 200 ms, and the maximum ratio was obtained at an interstimulus interval of approximately 40 ms. In addition, the PPF ratio decreased as the stimulus intensity increased (Fig. 5C). Therefore the stimulus intensity of 1.1 times greater than the minimal intensity (2.5–2.7 V) and the interstimulus interval of 40 ms were applied to evoke a pair of EPSCs, and the effects of ischemic exposure on the PPF ratio of the fast EPSCs were examined. Four minutes after starting the ischemic exposure, the amplitudes of the first and second EPSCs decreased by 83 ± 18\% (\(n = 20\), \(P < 0.01\)) and by 80 ± 16\% (\(n = 20\), \(P < 0.01\)) of the respective controls (Fig. 5, A and B). In contrast, the PPF ratio was 156 ± 24\% (\(n = 20\)) in the control medium (Fig. 5B), and significantly increased to 194 ± 40\% (\(n = 20\), \(P < 0.01\)) 4 min after the onset of ischemic exposure. The rise time and decay time constant (2.3 ± 0.6 ms and 7.6 ± 2.6 ms, respectively) of the first EPSC were not different from those (2.6 ± 0.9 ms and 7.3 ± 2.3 ms, respectively) of the second EPSC in the control medium. The rise time and decay time constant of the first and second EPSCs did not change during ischemic exposure. 8-CPT (0.3–10 \(\mu\)M) reversibly antagonized the reductions in the amplitudes of paired EPSCs and the enhancement of the PPF ratio during ischemic exposure (Fig. 5, A and B). In the presence of 8-CPT (0.3–10 \(\mu\)M), the PPF ratio (150 ± 21\%, \(n = 20\)) in the control was not significantly different from that (155 ± 19\%, \(n = 20\)) 4 min after the onset of ischemic exposure. These results suggest that, during in vitro ischemia, activation of A1 receptors on the axon terminals may reduce the probability of Glu release.

**In vitro ischemia did not change presynaptic volleys**

To assess the effects of ischemic exposure on the impulses of the afferent axon, a pair of presynaptic volleys and of subsequent field EPSPs (fEPSPs) were obtained by extracellular recording from proximal stratum radiatum of the CA1 region in the presence of bicuculline (10 \(\mu\)M). Four minutes from starting the ischemic exposure, the slopes of the first and...
second fEPSPs reversibly decreased by 77 ± 10% and by 72 ± 20% (n = 11) in comparison to the respective controls (Fig. 5A). The PPF ratio in the control medium was 149 ± 24% (n = 11), and the ratio 4 min after the onset of the ischemic exposure significantly increased to 181 ± 27% (n = 11, P, 0.05). The paired presynaptic volleys could be recorded in the presence of ionotropic glutamate receptor antagonists (CNQX, 20 µM and AP5, 100 µM; Fig. 5B). TTX (0.5 µM) abolished the presynaptic volleys (n = 11; Fig. 5B, middle trace), thus suggesting that the presynaptic volley reflects Na\(^+\)-dependent spikes of the afferent fibers. The subtraction of the trace recorded in the presence of CNQX (20 µM), AP5 (100 µM), and TTX (0.5 µM) from that in the presence of CNQX (20 µM) and AP5 (100 µM) using the AxoGraph program allowed us to distinguish the TTX-sensitive presynaptic volley from the stimulus artifact (Fig. 5B, left trace). The amplitudes of the first and second presynaptic volleys were 1.48 ± 0.55 mV (n = 11) and 1.49 ± 0.54 mV (n = 11), respectively. These presynaptic volleys did not significantly change following 4 min ischemic exposure (Fig. 5C); the amplitude of the first presynaptic volley was 1.43 ± 0.52 mV and the second was 1.44 ± 0.5 mV. These results support the idea that the depression of the paired fEPSPs by in vitro ischemia may be due to the activation of presynaptic A1 receptors, but not due to the depression of the presynaptic volley.

**DISCUSSION**

**In vitro ischemia-induced depression of the evoked fast EPSCs**

The present study demonstrates that evoked fast EPSCs were reversibly decreased by a brief exposure (4 min) to in vitro ischemia. The amplitude of the fast EPSCs decreased by approximately 85% of the control during in vitro ischemia. The rise time and decay time constant of the fast EPSCs did not change during in vitro ischemia, thus suggesting that the open and close time constants of the ionotropic Glu receptor channels for generating the evoked EPSC may not change. The inward currents produced by exogenous Glu application increased during in vitro ischemia. However, the amplitude of spontaneous miniature EPSCs did not change during in vitro ischemia. These results suggest that the depression of the fast EPSCs by in vitro ischemia is due to presynaptic mechanisms. The results on the amplitudes of the Glu-induced inward current and the miniature EPSCs seem to be contradictory. Energy deprivation applied by chemicals, such as iodoacetate (2 mM) and cyanide (1 mM), decreases net Glu uptake within 2–3 min in rat hippocampal slice cultures (Jabaudon et al. 2000). It is therefore highly possible that the slowing down of the Glu uptake during in vitro ischemia may augment the Glu-induced inward current, but not affect the very brief miniature EPSCs. Alternatively, it is also possible that the augmentation of the
Glu-induced inward current may be due to the suppression of the Glu uptake system mainly at extrasynaptic sites, but not at synaptic clefts. The depression of the fast EPSCs were reversibly inhibited by an A1 receptor antagonist, 8-CPT, in a dose-dependent manner (0.3–10 μM). The result indicates that the A1 receptor activation mediates the presynaptic inhibition of fast EPSCs by in vitro ischemia. This finding is comparable with the previous findings that showed A1 receptor antagonist, 8-CPT, to reversibly inhibit a decrease in the fEPSPs recorded extracellularly in the rat CA1 region following hypoxia (Doolette and Kerr 1995; Gribkoff and Bauman 1992; Gribkoff et al. 1990). Katchman and Hershkowitz (1993a,b) reported that the evoked fast EPSC and fast IPSC in CA1 neurons decrease during hypoxia, and A1 receptor antagonists only inhibit the hypoxia-induced decrease of the fast EPSC. Ischemia and hypoxia have been shown to increase extracellular adenosine in the in vivo brain (Winn et al. 1979) and in vitro hippocampal slice preparations (Fredholm et al. 1984; Latini et al. 1998), respectively. The present study, however, demonstrated that even in the presence of 8-CPT at high concentrations (1, 10 μM), in vitro ischemia depressed the amplitude of evoked fast EPSCs. In the presence of 8-CPT (1 μM), the maximal slope and half-width of nifedipine-resistant Ca2+ spikes in CA3 neurons decreased during in vitro ischemia. In CA1 neurons, L- and T-type Ca2+ currents are suppressed by anoxia (Krnjevic and Leblond 1989). The voltage-dependent Ca2+ channels for producing the fast EPSP and EPSC in CA1 neurons are, however, dihydropyridine-resistant, ω-conotoxin GVIA-sensitive (N-type) and ω-agatoxin IVA-sensitive (P/Q-type) Ca2+ channels (Higashi et al. 1990; Wheeler et al. 1994; Wu and Saggau 1994). In addition, Fujiwara et al. (1992) reported that the intracellular pH of mouse hippocampal slices decreases during in vitro ischemia. In isolated rat CA1 neurons, high-voltage–activated Ca2+ currents are depressed by low pH (pH 6.9–6.0) (Tombaugh and Somjen 1996). It may be possible that the decrease in pH during in vitro ischemia induces a reduction in the high-voltage–activated Ca2+ currents in afferent axon terminals. From the present results, in combination with previous findings, it is most likely that the remaining reduction of the fast EPSCs during in vitro ischemia in the presence of 8-CPT may be due to a direct depression of the nifedipine-resistant Ca2+ currents in afferent axon terminals onto CA1 neurons (also see the next section).

In vitro ischemia-induced enhancement of the PPF

The PPF ratio has been found to be inversely correlated with the release probability in CA1 neurons (Dobrunz and Stevens 1997; Manabe et al. 1993; Zucker 1989). During in vitro ischemia, the first and second EPSCs elicited by paired-pulse stimulation were decreased in amplitude, while the PPF ratio increased without affecting the pair of presynaptic volleys. Furthermore, the frequency of miniature EPSCs decreased 2
After the onset of in vitro ischemia, the release probability and/or the number of release sites for Glu decrease. In addition, the increase in the PPF ratio following in vitro ischemia was antagonized by 8-CPT (0.3–10 μM), suggesting that the augmentation of the PPF is mediated by presynaptic A1 receptors. Nevertheless, the frequency of the miniature EPSCs increased 4 min after the onset of in vitro ischemia. This result is comparable with previous findings that in CA1 neurons in vitro ischemia and anoxia increase the frequencies of spontaneous EPSPs (Tanaka et al. 1997) and miniature EPSCs (Katchman and Hershkowitz 1993b). Katchman and Hershkowitz (1993b) demonstrated that the increase in the frequency of miniature EPSCs by anoxia results from the Ca\(^{2+}\) mobilization from the dantrolene-sensitive intracellular stores. The nerve terminals of the CA3 neurons mainly project to apical dendrites in the stratum radiatum of the CA1 region. The intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in the stratum radiatum increases during ischemic exposure (Tanaka et al. 1997). It is therefore possible that the raised [Ca\(^{2+}\)]\(_i\) in the nerve terminals may increase the frequency of miniature EPSCs and decrease the Ca\(^{2+}\) influx into the nerve terminals.

In conclusion, in vitro ischemia for a short period resulted in a decrease in the evoked fast EPSCs. Such a decrease may mainly be due to the activation of presynaptic A1 receptors and also may partially be caused by a direct reduction of nifedipine-resistant Ca\(^{2+}\) currents.

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