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Characterization of the anoxia-induced long-term synaptic potentiation in area CA1 of the rat hippocampus

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1 The purpose of the present study was to characterize the mechanisms underlying the anoxia-induced long-term potentiation (LTP) of glutamatergic synaptic transmission in the CA1 region of rat hippocampus by use of intracellular recordings *in vitro*.

2 In response to superfusion of an anoxic medium equilibrated with 95% N_2–5% CO_2, the initial slope (measured within 3 ms from the onset of the synaptic response) of the excitatory postsynaptic potential (e.p.s.p.) generated in the hippocampal CA1 neurones by stimulation of Schaffer collateral-commissural afferent pathway was significantly decreased by $91.3 \pm 4.9\%$ ($n=10$) within 10 min of the anoxic episode. The reduction of the initial slope of the e.p.s.p. was accompanied by a transient membrane hyperpolarization followed by a sustained depolarization (10.8 ± 1.7 mV, $n=10$), along with a reduction in membrane input resistance ($69.3 \pm 4.8\%$ of control, $n=10$). On return to reoxygenated medium, the e.p.s.p. slope returned to the control value within 8–10 min and was subsequently and progessively potentiated to reach a plateau ($195.6 \pm 14.7\%$ of control, $n=10$) 15–20 min after return to control ACSF. This anoxic episode-induced persistent potentiation of synaptic transmission lasted for more than 1 h and was termed anoxic LTP.

3 The anoxic episode induced a persistent potentiation of the initial slopes of both pharmacologically isolated α-amino-3-hydroxy-5-methyl-4-isoxazola-propionate (AMPA) receptor-mediated e.p.s.p. (e.p.s.p.$_{AMPA}$) and *N*-methyl-D-aspartate (NMDA) receptor-mediated e.p.s.p. (e.p.s.p.$_{NMDA}$) with a similar time course and magnitude. The sensitivity of postsynaptic neurones to NMDA (10 μM), but not to AMPA (10 μM) was also persistently potentiated following the anoxic episode. In addition, the anoxia-induced LTP of the initial slope of e.p.s.p.$_{AMPA}$ was accompanied by a decrease in the magnitude of paired-pulse facilitation (PPF; from 106.8 ± 17.6 to $46.6 \pm 18.4\%$, $n=6$), a phenomenon which was associated with presynaptic transmitter release mechanisms.

4 The induction of the anoxic LTP is dependent on the extracellular Ca^{2+} concentration. The induction of the anoxic LTP was completely abolished when the external Ca^{2+} was removed and substituted with equimolar Mg^{2+}. Moreover, the anoxic LTP was completely abolished in neurones intracellularly recorded with Ca^{2+} chelator *bis*-(O-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA, 500 mM).

5 Occlusion experiments were performed to examine whether the sustained enhancement of the initial slope of the e.p.s.p. produced by tetanic stimulation and the anoxic episode share common cellular mechanisms. Three episodes of tetanic stimulation were delivered to saturate the LTP, following which a long period (15 min) of anoxia failed to cause a further potentiation of the initial slope of the e.p.s.p. Similarly, prior induction of anoxic LTP also significantly attenuated the subsequent synaptic potentiation induced by a high-frequency tetanic stimulation (100 Hz for 1 s duration). These data imply that these two forms of synaptic plasticity may share a common cellular mechanism.

6 These results provide strong evidence that the generation of the anoxia-induced LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus probably involves both of the presynaptic and postsynaptic loci. The mechanisms underlying the persistent potentiation are likely to be attributable to an enhancement of presynaptic glutamate release and a selective upregulation of postsynaptic NMDA receptor-mediated synaptic response through the Ca^{2+}-dependent processes.

Keywords: Anoxia-induced long-term potentiation (anoxic LTP); *N*-methyl-D-aspartate receptor (NMDA receptor); non-NMDA receptor; long-term potentiation (LTP); paired-pulse facilitation (PPF); hippocampus

Introduction

It is well known that the brain requires a continuous supply of oxygen to maintain the normal function and viability. Higher brain functions such as consciousness and purposeful behaviour are lost in the absence of oxygen supply, but these will return with timely reoxygenation (Leblond & Krnjevic, 1989). The brain is critically dependent on its blood flow for a continuous supply of oxygen. Oxygen to the brain can be cut-off by a loss of blood flow (ischaemia), for example owing to cardiac arrest or intracranial vessel occlusion, or by insufficient oxygen in the blood (hypoxia) induced by perinatal asphyxia or carbon monoxide poisoning (Martin *et al*., 1994). In man and experimental animals, it has been shown that a short period of hypoxia or ischaemia can trigger a cascade of events leading to neuronal damage and then to death. In addition, a number of processes have been proposed to contribute to the hypoxia- or ischaemia-induced neuronal injury. Recent evidence has suggested that the neuronal injury after transient hypoxic or ischaemic insults is a result of excessive facilitation of glutamate release from nerve terminals during the period of the hypoxic or ischaemic insult, which causes excessive activation of N-methyl-D-aspartate (NMDA)-type glutamatergic receptors, and subsequent massive influx of Ca^{2+} through NMDA receptor-linked ion channels (Choi, 1990; Zini *et al*., 1993; Hori & Carpenter, 1994). This hypothesis has been confirmed by recent microdialysis studies showing that the extracellular levels of glutamate and aspartate increase from the normal, low micromolar values, up to several hundred micromolar during the period of hypoxic or ischaemic insult (Benveniste *et al*., 1984; Globus *et al*., 1988; Choi, 1990; Young

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et al., 1993). Additionally, the NMDA receptor antagonists aminophosphonovaleric acid (APV) and MK-801 have been shown to protect effectively against the neuronal damage induced by several models of focal brain ischaemia *in vivo* and *in vitro* (Choi, 1988; Kass *et al*., 1989). These facts suggest that the parenchyma approach to the protective strategies against hypoxia or ischaemia-induced neuronal damage can be generally countered by attending specifically to brain excitatory synaptic transmission.

Neuronal death following hypoxic or ischaemic insults can be either immediate, such as within the neurones of the infarct core, or delayed, such as neurones in the penumbra. In man and experimental animals, the CA1 region of the hippocampus has been found to be the most vulnerable brain region to ischaemic or hypoxic injury (Chang *et al*., 1989; Choi, 1990). *In vivo* models of transient global ischaemia followed by reperfusion have demonstrated that the selective neuronal death of the pyramidal neurones in the CA1 region of the rat hippocampus occur 1–3 days after insult, while other neurones in the same region remain intact (Petito *et al*., 1987; Chang *et al*., 1989). The mechanisms responsible for delayed neuronal death of the vulnerable hippocampal CA1 pyramidal neurones is unclear. It was therefore of interest to study in detail the physiological events which initiate the delayed death of hippocampal CA1 pyramidal neurones after transient ischaemic or hypoxic injury. Some investigators have shown that the spontaneous neuronal activity and the excitability of the hippocampal CA1 neurones are increased after reperfusion, by use of extracellular recording techniques *in vivo* (Suzuki *et al*., 1983; Doolete & Kerr, 1995). In addition, the electrophysiological properties of neuronal perikarya and synaptic transmission after brief cerebral ischaemia may also participate in the pathophysiological mechanisms that cause acute or delayed selective neuronal injury (Xu & Pulsinelli, 1996). Using intracellular and extracellular recording techniques *in vitro*, Ben-Ari and his colleagues have found that there is a loss of glutamatergic synaptic transmission during a short period (2–3 min) of the anoxic-aglycaemic episode, whereas the CA1 pyramidal neurones showed a long-term synaptic potentiation during the period of reoxygenation (Crépel *et al*., 1993; Gozlan *et al*., 1994; Hammond *et al*., 1994). This novel form of long-term potentiation (LTP) of synaptic transmission generated by a short period of anoxic-aglycaemic episode has been termed anoxic LTP, and this phenomenon has been proposed as the early marker of delayed neuronal death induced by a transient ischaemic insult in the hippocampal CA1 neurones (Hammond *et al*., 1994). However, such anoxic LTP is poorly characterized. For instance, some studies have suggested that this phenomenon is essentially associated only with an enhancement of postsynaptic NMDA receptor-mediated synaptic responses, resulting in an increase in the excitability of pyramidal neurones (Crépel *et al*., 1993; Gozlan *et al*., 1994; Hammond *et al*., 1994). Whether an increase in the presynaptic glutamate release mechanism also contributes to the generation of the anoxic LTP is still unclear. A detailed investigation of the mechanisms underlying anoxic LTP may help us to clarify the precise mechanisms of the delayed death of the hippocampal CA1 pyramidal neurones after a transient ischaemic or hypoxic insult. Hence, the aim of the present study was to determine whether the induction and maintenance of the anoxic LTP is only associated with postsynaptic mechanisms, such as enhancement of the postsynaptic NMDA receptor-mediated synaptic response, or whether there is a concomitant increase of presynaptic glutamate release mechanisms. Moreover, it is also of interest to examine further whether the anoxic LTP can interact with the induction of tetanic LTP, which is a prolonged form of synaptic plasticity believed to be involved in learning and memory (Malenka & Nicoll, 1993; McNaughton, 1993). To address these two issues, the *in vitro* hippocampal slice has been used as a model system to study the effect of a long period of the anoxic episode (15 min) on glutamatergic synaptic transmission in the CA1 region of rat hippocampus, by use of intracellular recording techniques.

Methods

Slice preparation and intracellular recordings

Hippocampal slices (500 μm thick) were isolated from male adult Sprague-Dawley rats (150–200 g) for intracellular recordings by the procedure described previously (Hsu, 1996; Hsu & Kan, 1996; Huang *et al*., 1996). In brief, the rats were decapitated, and transverse slices were cut from a tissue block of the brain with a Vibroslice (Campden Instruments, Silbey, U.K.). The slices were placed in a beaker of artificial cerebral spinal fluid (ACSF) oxygenated with 95% O_2–5% CO_2 and kept at room temperature for at least 1 h before recording. The composition of the ACSF solution was (in mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11 at pH 7.4 and equilibrated with 95% O_2–5% CO_2. In some experiments, $CaCl_2$ was totally replaced by equimolar $MgCl_2$.

A single slice was then transferred to the recording chamber, in which it was held submerged between two nylon nets and maintained at 32 ± 1°C. The chamber consisted of a circular well of a low volume (1–2 ml) and was perfused constantly at a rate of 2–3 ml min^{-1}. A bipolar stimulating electrode (Kopf Instruments SNE-200X, Bern, Germany) was positioned in the stratum radiatum to stimulate the Schaffer collateral-commissural afferent pathway. Orthodromic stimuli were delivered with monophasic constant-voltage pulses at 0.033 Hz from a Grass stimulator with an isolation unit. The stimulus intensity was adjusted to subthreshold for action potential initiation. The strength of synaptic transmission was quantified by measuring the initial slope of e.p.s.ps (measured within the 3 ms from onset of the synaptic responses). As a contitioning tetanus, a single train of tetanic stimulation (100 Hz for 1 s duration) was used to evoke tetanic LTP. Intracellular recordings were made from CA1 pyramidal neurones by use of glass microelectrodes filled with 4 M potassium acetate (60–80 MΩ). Microelectrodes were pulled from microfibre 1.0 mm capillary tubing on a Brown-Flaming electrode puller (Sutter Instruments, San Rafael, CA, U.S.A). Electrical signals were amplified by an Axoclamp-2B amplifier (Axon Instruments, Foster, CA, U.S.A.) and an IBM 486-based computer with pCLAMP software (Version 6.0.3, Axon Instruments, Foster, CA, U.S.A.) was used to on-line acquire and analyse the data. In experiments in which intracellular calcium was buffered, intracellular recording microelectrodes were also filled with *bis*(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA, 500 mM). BAPTA, dissolved in 4 M potassium acetate, was applied intracellularly by allowing it to leak from the microelectrode for at least 40 min before the delivery of the anoxic episode, according to the method of Xie *et al*. (1992). The effectiveness of BAPTA treatment was confirmed by the finding that the afterhyperpolarizations (AHPs) evoked by depolarizing current pulse injection (0.5–0.6 nA, 200 ms) were abolished. In contrast, without BAPTA in the recording microelectrode, AHPs were typically observed for more than 1 h.

Drug application

All drugs were applied by dissolving them to the desired final concentrations in the ACSF and by switching the perfusion from control ACSF to drug-containing ACSF. Appropriate stock solutions of drugs were made and diluted with ACSF just before application. All stock solutions of drugs except 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were made up in deionized water and stored at −20°C before use. CNQX was dissolved in a dimethylsulphoxide (DMSO) stock solution and kept frozen until the day of experiment. The concentration of DMSO in the perfusion medium was about 0.1%, this did not affect either the active or the passive electrophysiological properties of the recording neurones ($n = 3$) (Hsu & Kan, 1996; Huang *et al*., 1996).

The anoxic episodes were produced by switching to ACSF solution equilibrated with 95% N_2–5% CO_2. The exchange of solution experienced a delay of 0.5–1 min before the new solution reached the recording chamber. DL-2-amino-phosphno-valerate (DL-APV), 6-cyano-7-nitroquinoxaline-2-3-dione (CNQX), N-methyl-D-aspartate (NMDA), (±)-α-amino-3-hydroxy-5-methyllisoxazole-4-propionic acid (AMPA), picrotoxin and *bis*-(-o-amino-phenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA) were purchased from Research Biochemicals Inc. (Natick, MA, U.S.A.).

Statistical analysis

Results are expressed as mean ± s.e.mean. The significance of difference was evaluated by Student's two-tailed paired or unpaired *t* test where appropriate. Numbers of experiments are indicated by *n*. Probability values (*P*) of less than 0.05 were considered to be significant.

Results

The effects of anoxia on neuronal excitability and excitatory synaptic transmission were studied in the CA1 region of rat hippocampal slices by use of intracellular recording techniques. The data presented here were obtained from 96 CA1 pyramidal neurones with stable membrane potentials more negative than −55 mV and action potential amplitudes greater than 70 mV. A random sample of 42 neurones had a mean input resistance of 42.3 ± 2.7 MΩ with resting membrane potential (RMP) of −64.5 ± 2.9 mV.

Effects of anoxia on the e.p.s.ps

Orthodromic stimulation of the Schaffer collateral-commissural afferent pathway evoked an excitatory postsynaptic potential (e.p.s.p.) in hippocampal CA1 neurones. In the initial set of experiments, we tested the effect of the anoxic insult on neuronal excitability and excitatory synaptic transmission in the slices. A representative record, shown in Figure 1a, illustrates the general sequence of changes in the initial slope of e.p.s.p. and the passive electrophysiological properties of the hippocampal CA1 neurones due to an anoxic episode. The initial slope of the e.p.s.p. was reduced to 8.7 ± 4.9% ($n = 10$) within 10 min after the onset of anoxia. On return to reoxygenated medium, the e.p.s.p. slope returned to control value within 8–10 min and was subsequently and progressively potentiated to reach a plateau 15–20 min after return to oxygen. This post-anoxic synaptic potentiation lasted for more than 1 h and was observed in 8 out of 10 slices tested in this set of experiments (Figure 1b). The degree of this long-lasting synaptic potentiation following the anoxic episodes varied in different cells tested, ranging from 54 to 138% (mean % of control: 95.6 ± 14.7% ($n = 10$), $P < 0.001$). In addition, the inhibition of the initial slope of e.p.s.p. during the period of the anoxic episode was accompanied by an alteration of the passive electrophysiological properties of the recorded neurones. There was a transient membrane hyperpolarization followed by a sustained depolarization and a reduction in the neuronal membrane input resistance (Figure 1b). The peak amplitude of the depolarization was 10.8 ± 1.7 mV ($n = 10$), while the input resistance at the peak of depolarization was reduced to 30.7 ± 4.8% (range 25–42%, $n = 10$). However, the neuronal resting membrane potential and the membrane input resistance began to recover within 5 min of reintroduction of control ACSF and full recovery took 10–15 min (Figure 1b). These results are consistent with the previous findings described by Crépel *et al.* (1993), who found that a short period (2–3 min) of the anoxic-aglycaemic episode can result in long-term potentiation of the excitatory synaptic transmission in the CA1 region of rat hippocampus.

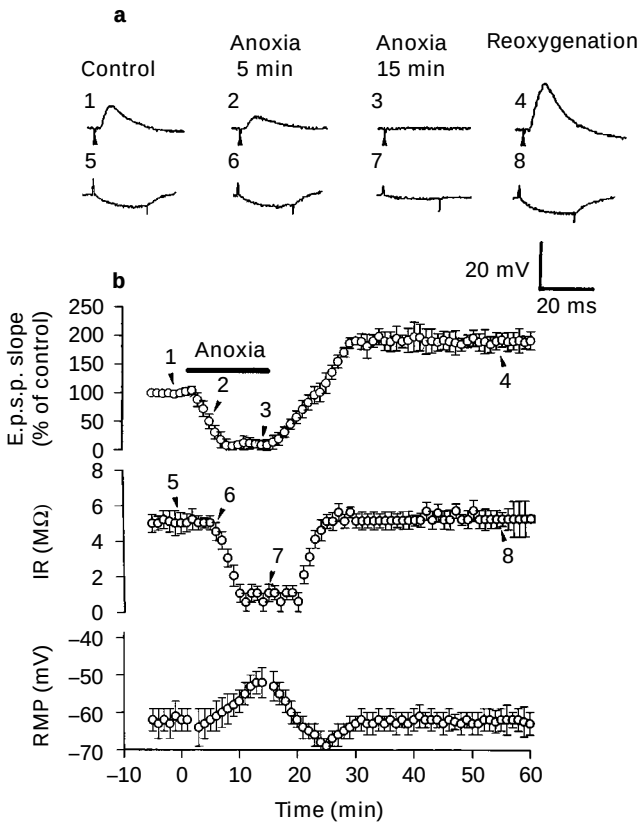

Figure 1 Effect of an anoxic episode on synaptic transmission. (a) A depolarizing excitatory postsynaptic potential (e.p.s.p.) evoked by stimulation of Schaffer collateral-commissural afferent pathway. A hyperpolarizing current pulse (−0.2 nA, 50 ms) was injected through the recording electrode to monitor the neuronal membrane input resistance. Representative traces showing the e.p.s.p. recorded before (1), during (2 and 3) and 40 min after (4) the anoxic episode (15 min duration). Note that the e.p.s.p. was fully blocked within 10 min after the onset of anoxia. On return to reoxygenated medium the e.p.s.p. returned to a persistently potentiated level. The resting membrane potential of this recorded neurone was −62 mV. (b) Time courses of effect of the anoxic episode on the initial slope of e.p.s.p., membrane input resistance (IR) and resting membrane potential (RMP) ($n = 10$). During the period of the anoxic episode, the depression of the initial slope of the e.p.s.p. was accompanied by a substantial reduction in membrane resistance and a small transient membrane hyperpolarization followed by an increasing depolarization. The neuronal membrane input resistance and resting membrane potential began to recover within 5 min on return to reoxygenated medium and full recovery took 10–15 min. Solid triangles represent the point of synaptic stimulation. Bar denotes the period of delivery of the anoxic episode.

Effects of anoxia on the AMPA- and NMDA-mediated component of e.p.s.ps

Several lines of evidence have indicated that the e.p.s.p. evoked by stimulation of the Schaffer collateral-commissural afferent pathway is mediated by AMPA and NMDA receptors on the hippocampal CA1 neurones (Collingridge *et al.*, 1988; Andreasen *et al.*, 1989; Hestrin *et al.*, 1990; Hsu, 1996; Hsu & Kan, 1996). The next set of experiments was to address which subtype of glutamate receptor was responsible for the anoxia-induced long-term potentiation of synaptic transmission in the hippocampal CA1 neurones. In first series of experiments the effect of anoxia on a pure AMPA receptor-mediated e.p.s.p. ($e.p.s.p._{AMPA}$) was examined. To obtain such an $e.p.s.p._{AMPA}$ the perfusion of ACSF contained a high (50 μM) concentration of the NMDA receptor antagonist DL-APV to abolish the NMDA receptor-mediated component of the e.p.s.p. and the γ-aminobutyric acid ($GABA_A$) receptor antagonist picrotoxin (50 μM) to abolish the $GABA_A$ receptor-mediated inhibitory

postsynaptic potential (i.p.s.p.) (Hanse & Gustafsson, 1995). The remaining e.p.s.p. was completely blocked by application of the AMPA receptor antagonist CNQX (10 μM), indicating that it is mediated by the activation of AMPA receptors. As shown in Figure 2, the intensity of the test stimulus was chosen to evoke an e.p.s.p.$_{AMPA}$ amplitude 50% of the maximum response. Following the switch from normoxic to anoxic ACSF, the initial slope of e.p.s.p.$_{AMPA}$ was maximally depressed within 10 min ($n=10$, mean maximal depression in 10 neurones was by 81.4 ± 7.9% during 15 min of anoxia). On return to control ACSF the initial slope of the e.p.s.p.$_{AMPA}$ returned to control values within 10–15 min and was subsequently and persistently potentiated ($\geqslant$1 h, $n=8$ cells out of 10, mean potentiation in 10 neurones was by 84.1 ± 11.2% 40 min following 15 min of anoxia) (Figure 2). In addition, the anoxia-induced LTP of the initial slope of the e.p.s.p.$_{AMPA}$ had a similar time course and magnitude of the anoxia-induced LTP of the composite e.p.s.p.

It has been shown that the induction and maintenance of the anoxic LTP of glutamatergic synaptic transmission in rat hippocampal CA1 neurones is due to a persistent enhancement of the NMDA receptor-mediated synaptic transmission (Crépel *et al*., 1993; Gozlan *et al*., 1994; Crépel & Ben-Ari, 1996). In order to explore whether the NMDA receptor-mediated component of the synaptic response can express the anoxic LTP, we examined the effect of the anoxic episode on the NMDA receptor-mediated component of the e.p.s.p. (e.p.s.p.$_{NMDA}$). To isolate the pure e.p.s.p.$_{NMDA}$, experiments were carried out in the presence of both the AMPA receptor antagonist CNQX (10 μM) and the $GABA_A$ receptor antagonist picrotoxin (50 μM), leaving an isolated NMDA receptor-mediated e.p.s.p. Under this condition the remaining e.p.s.p. had a long rise time and was completely blocked by application of the NMDA receptor antagonist DL-APV (50 μM), indicating it was mediated by NMDA receptors. As shown in Figure 3, the anoxic episode strongly and progressively depressed the initial slope of e.p.s.p.$_{NMDA}$ to a steady level (mean maximal depression in 11 neurones tested was by 8.39 ± 2.1% during 15 min of anoxia). On return to reoxygenated ACSF, the initial slope of e.p.s.p.$_{NMDA}$ progressively returned to the control values within 8–10 min and was progressively potentiated to reach a stable level after 10–15 min ($n=9$ cells out of 11; mean potentiation in 11 neurones was 76.8 ± 15.6% 40 min following 15 min of anoxia) (Figure 3). The time course and the magnitude of the anoxia-induced LTP of e.p.s.p.$_{NMDA}$ is similar to the anoxia-induced LTP of the composite e.p.s.p. These results are consistent with the previous findings of Crépel *et al*. (1993), who showed that the NMDA-receptor component of the synaptic response is essential for the induction and maintenance of the anoxic-aglycaemic episodes-induced LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus.

Postsynaptic action of anoxia

To determine whether the anoxia-induced LTP of the glutamatergic synaptic transmission in rat hippocampal CA1 neurones was mediated by a pre- or postsynaptic mechanism, we next examined the effect of anoxia on the postsynaptic responses to exogenously applied AMPA and NMDA. The experiments were performed in the presence of tetrodotoxin (0.5 μM). As illustrated in Figure 4, application of AMPA (10 μM, 1 min) or NMDA (10 μM, 1 min) produced a membrane depolarization, 15.8 ± 2.1 mV ($n=6$) and 17.6 ± 3.1 mV ($n=6$), respectively. During the anoxic episode, both of the AMPA- and NMDA-induced membrane depolarizations were significantly depressed. The mean amplitude of AMPA- or NMDA- induced membrane depolarizations was reduced to 69.8 ± 3.9% ($n=6$, $P<0.01$) and 72.4 ± 5.3% ($n=6$), $P<0.01$), respectively. On return to reoxygenated ACSF for 30 min, the AMPA-induced membrane depolarization returned to the control value ($16.4\pm$ 2.4 mV, $P>0.1$, $n=6$). However, a persistent potentiation of NMDA-induced membrane depolarization (22.1 ± 1.2 mV, $P<0.05$, $n=6$) was observed 30 min

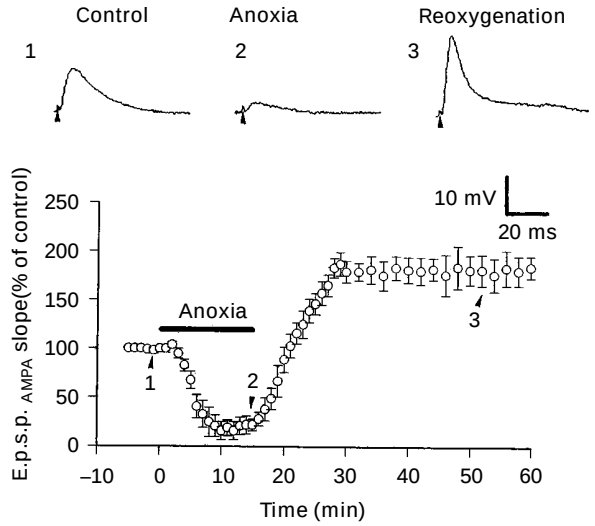

Figure 2 Pharmacologically isolated AMPA receptor-mediated e.p.s.p. is persistently potentiated following the anoxic episode. The AMPA receptor-mediated e.p.s.p. (e.p.s.p.$_{AMPA}$) was recorded in the continuous presence of NMDA receptor antagonist DL-APV (50 μM) and $GABA_A$ receptor antagonist picrotoxin (50 μM) in the bath. Representative traces showing the e.p.s.p.$_{AMPA}$ recorded before (1), during (2) and 40 min following (3) 15 min of the anoxic episode (evoked at $V_m=-61$ mV). The percentage change of the initial slope of e.p.s.p.$_{AMPA}$ ($n=10$) was plotted as a function of time. Note that the e.p.s.p.$_{AMPA}$ slope was progressively depressed during the anoxic episode. Following reoxygenation, e.p.s.p.$_{AMPA}$ recovered to a persistently potentiated level. Solid triangles represent the point of synaptic stimulation. Bar denotes the period of delivery of the anoxic episode.

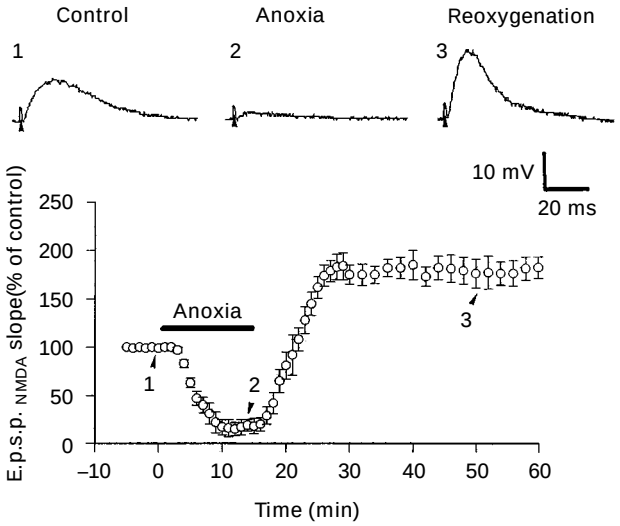

Figure 3 Pharmacologically isolated NMDA receptor-mediated e.p.s.p. is persistently potentiated following the anoxic episode. The NMDA receptor-mediated e.p.s.p. (e.p.s.p.$_{NMDA}$) was recorded in the continuous presence of non-NMDA receptor antagonist CNQX (10 μM) and $GABA_A$ receptor antagonist picrotoxin (50 μM). The percentage change of the initial slope of e.p.s.p.$_{NMDA}$ ($n=11$) was plotted as a function of time. Representative traces showing the e.p.s.p.$_{NMDA}$ before (1), during (2), and 40 min after (3) the anoxic episode (15 min duration; evoked at $V_m=-65$ mV). During anoxia, the e.p.s.p.$_{NMDA}$ was progressively decreased to a steady state. On return to reoxygenated ACSF the e.p.s.p.$_{NMDA}$ recovered to a persistently potentiated level. Bar denotes the period of delivery of the anoxic episode. Solid triangles represent the point of synaptic stimulation.

following 15 min of the anoxic episode. We also studied the influence of intracellular Ca^{2+} concentration on the induction of anoxia-induced persistent enhancement of the NMDA-induced membrane depolarization. Before delivery of the anoxic episode, intracellular Ca^{2+} was buffered by intracellular application of the Ca^{2+} chelator BAPTA (500 mM) through the intracellular recording microelectrodes. In all 3 neurones tested, BAPTA completely blocked the induction of the anoxia-induced persistent potentiation of the NMDA-induced membrane depolarization. Thirty min after the reoxygenation, in BAPTA-loaded cells, the amplitudes of the NMDA-induced membrane depolarization were not significantly changed ($104.7 \pm 5.2\%$ of control, $P > 0.05$, $n = 3$; data not shown). These results indicate that the anoxic episode under our experimental condition could selectively induce a persistent up-regulation of the postsynaptic NMDA receptor-mediated synaptic response in the hippocampal CA1 neurones in an intracellular free Ca^{2+} -dependent manner.

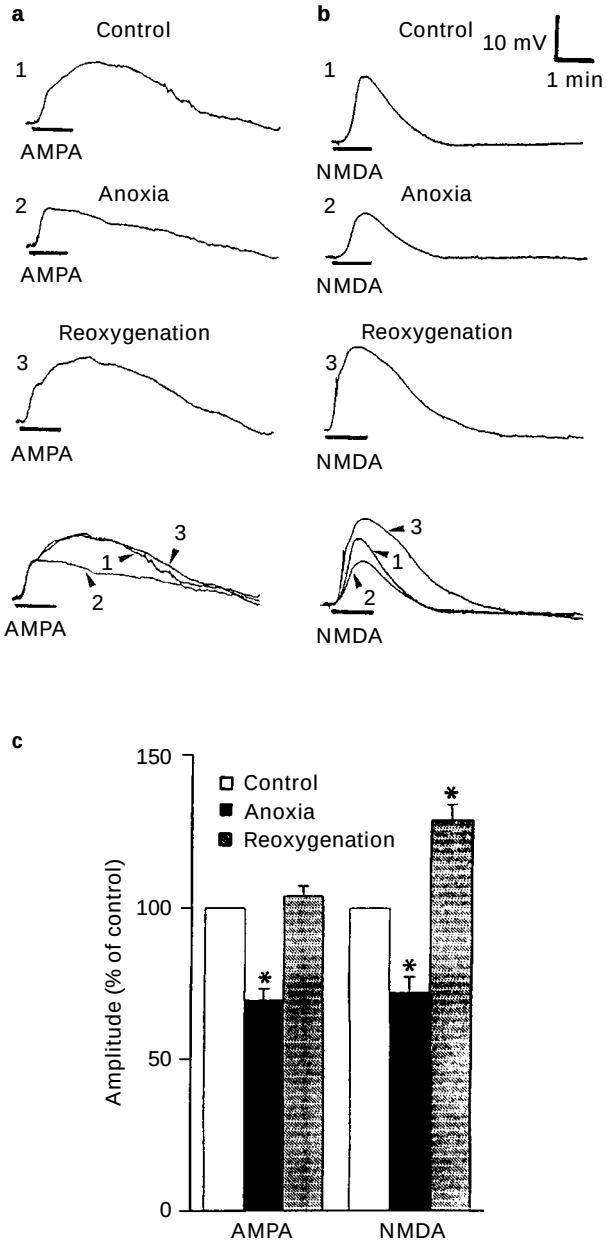

Figure 4 The anoxic episode selectively induces a persistent potentiation of the NMDA-induced membrane depolarization. Superfusion of either (a) AMPA (10 μM, 1 min) or (b) NMDA (10 μM, 1 min) evoked a membrane depolarization. From top to bottom are shown before, during, 30 min following 15 min of the anoxic episode and superimposed traces, respectively. During the period of the anoxic episode, both the AMPA- and DMDA-induced membrane depolarizations were significantly reduced. On return to reoxygenated medium for 30 min, the AMPA-induced membrane depolarization returned to the control baseline. However, a persistent potentiation of NMDA-induced membrane depolarization was observed 30 min after the return to oxygen. The anoxic episode selectively induced a long-term potentiation of postsynaptic NMDA receptor-mediated response. (c) The average percentage changes of either AMPA (10 μM)- or NMDA (10 μM)-induced membrane depolarization before, during and 30 min after the anoxia. The experiments were performed in the presence of tetrodotoxin (0.5 μM). Bars denote the periods of drug applications. Representative traces shown in (a) and (b) were taken from different pyramidal cells of different slices (evoked at $V_m = -63$ mV). Data are expressed as mean $\pm$ s.e.mean. $^*P < 0.05$ as compared with the respective control baseline.

Effects of the anoxic LTP on the paired-pulse facilitation (PPF) of AMPA receptor-mediated e.p.s.ps

Because the magnitude and the time course of persistent potentiation of the slope of e.p.s.p.$_{NMDA}$ and e.p.s.p.$_{AMPA}$ induced by the anoxic episode were similar, we next examined whether the anoxia-induced LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus resulted from an enhancement of glutamate release from presynaptic nerve terminals. To address this question, we examined the effect of anoxia on the paired-pulse facilitation (PPF) of the pharmacologically isolated e.p.s.p.$_{AMPA}$, a phenomenon which is associated with the presynaptic neurotransmitter release mechanisms (Clark *et al*., 1994). When the excitatory afferents to the hippocampal CA1 neurones are stimulated twice with a short inter-pulse interval, the synaptic response to the second stimulus is generally facilitated in relation to the initial stimulus (Dunwiddie & Hass, 1985; Hsu, 1996). This phenomenon is called PPF and is attributed an increase in the amount of transmitter released by the second stimulus (Manabe *et al*., 1993). On the other hand, the manipulation of presynaptic transmitter release mechanism may result in a change in the magnitude of the PPF ratio (Manabe *et al*., 1993; Schulz *et al*., 1994). If the anoxia-induced LTP of the synaptic response involves presynaptic mechanisms, then it might be associated with an alteration of the PPF ratio. Alternatively, if the anoxia-induced LTP of the synaptic response involves only postsynaptic mechanisms, then the PPF ratio should be relatively unaffected. This hypothesis was tested by comparing the magnitude of PPF ratio of the initial slope of e.p.s.p.$_{AMPA}$ before, during and after the anoxic episode. Pharmacologically isolated AMPA receptor-mediated synaptic responses (e.p.s.p.$_{AMPA}$) to a pair of stimuli were recorded with an inter-pulse interval of 60 ms (Figure 5a), and the PPF ratio is expressed as the initial slope of the second e.p.s.p.$_{AMPA}$ compared with the first. As shown in the Figure 5a and c, the second response was $106.8 \pm 17.6\%$ ($n = 6$) larger than the first response in the control condition. During the period of anoxia, the initial slope of the first e.p.s.p.$_{AMPA}$ was reduced by $89.8 \pm 6.2\%$, but the PPF ratio was significantly enhanced from 206.8 ± 17.6 to $268.7 \pm 17.6\%$ ($n = 6$). On return to reoxygenated ACSF medium, the e.p.s.p.$_{AMPA}$ slope returned to the control baseline and was subsequently and persistently potentiated for a long time ($\geqslant 1$ h), which was accompanied by a decrease in the magnitude of PPF ratio from 206.8 ± 17.6 to $146.6 \pm 18.4\%$ ($n = 6$) (Figure 5a and c). Figure 5d is a summary of 6 neurones tested in which the change in the initial slope of e.p.s.p.$_{AMPA}$ during and 30 min following 15 min of anoxic episode was plotted on the x-axis and the change in PPF ratio during and 30 min following 15 min of anoxic episode was plotted on the y-axis. It is clear that in all 6 neurones tested, the PPF ratio of e.p.s.p.$_{AMPA}$ slope was increased during the anoxic episode and, in contrast, the ratio of PPF was decreased 30 min after the anoxia. When the stimulus intensity was increased to counteract the direct depressant effect of anoxia on synaptic transmission, the enhancement effect of anoxia on the PPF ratio of e.p.s.p.$_{AMPA}$ slope was significantly reduced at high stimulus intensity from 61.9 ± 6.8 ($n = 6$) to

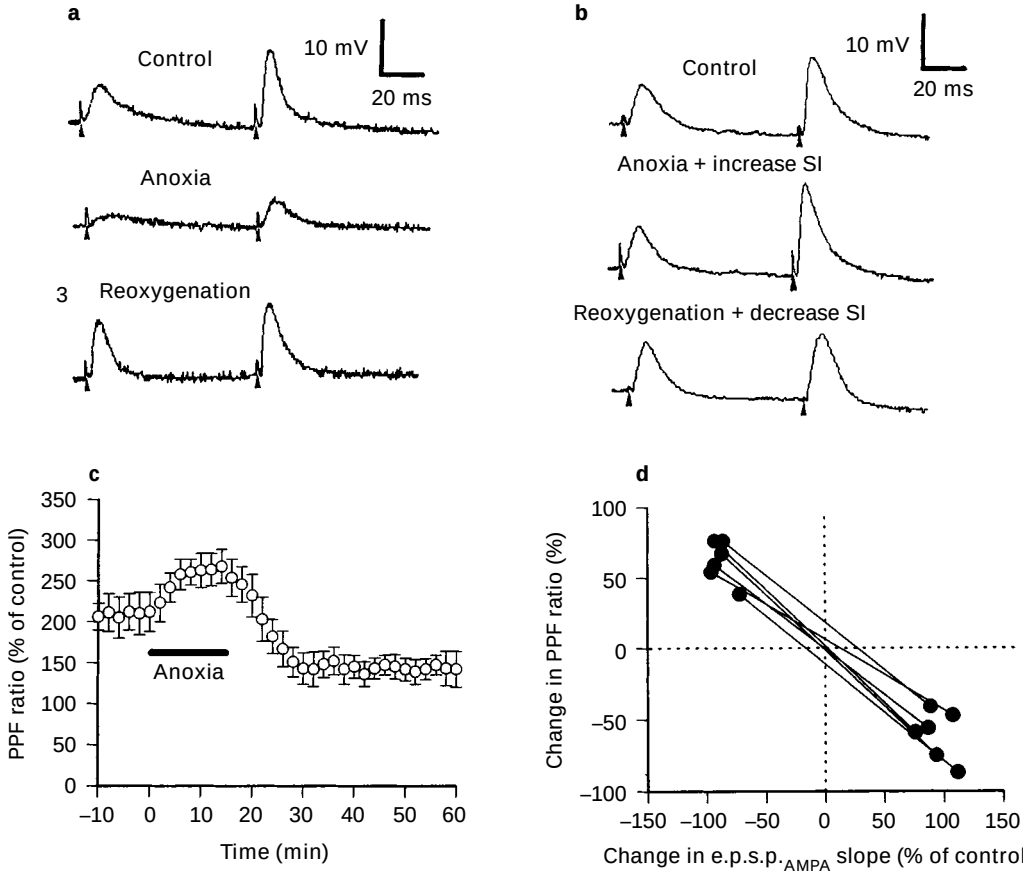

Figure 5 Anoxic LTP decreased the PPF of e.p.s.p.$_{AMPA}$. (a) Representative traces showing the pharmacologically isolated e.p.s.p.$_{AMPA}$ in the continuous presence of DL-APV (50 μM) and picrotoxin (50 μM) in the perfusion medium evoked by pair stimuli (60 ms inter-pulse interval) recorded before, during, and 30 min after the anoxic episode (15 min duration; evoked at $V_m = -61$ mV). (b) The enhancement of PPF ratio of the initial slope of e.p.s.p.$_{AMPA}$ was significantly reduced when the stimulus intensity (SI) was increased to counteract the depressant effect of anoxia on the synaptic transmission. Similarly, the inhibition of PPF of e.p.s.p. $_{AMPA}$ slope during the period of reoxygenation was also significantly decreased when the SI was reduced to counteract the enhancement of reoxygenation on the synaptic response. Similar results were also observed in another 3 neurones. (c) The percentage change of PPF ratio of the initial slope of e.p.s.p.$_{AMPA}$ plotted as a function of time. During anoxia, the e.p.s.p.$_{AMPA}$ slope was decreased, but the PPF ratio was significantly enhanced. On return to reoxygenated medium, the initial slope of the e.p.s.p. recovered to a persisent potentiated level which was accompanied by a decrease in PPF ratio. (d) The change in the initial slope of the e.p.s.p.$_{AMPA}$ during and 30 min after the 15 min of anoxic episode was plotted on the x-axis, and the change in PPF ratio was plotted on the y-axis. The change in PPF ratio of the initial slope of e.p.s.p.$_{AMPA}$ was calculated as the final PPF minus initial PPF. In all 6 neurones tested, the PPF ratio was increased during the period of the anoxic episode and, in contrast, the PPF ratio was decreased 30 min after reintroduction of the reoxygenated medium (evoked at $V_m = -61.3 \pm 3.7$ mV). Bar denotes the period of delivery of the anoxic episode. Solid triangles in (a) and (b) represent the point of synaptic stimulation.

$20.8 \pm 5.7\%$ ($n=4$).When the stimulus intensity was decreased to counteract the enhancement effect of reoxygenation on the synaptic responses, the inhibitory effect of reoxygenation on the PPF ratio of the initial slope of e.p.s.p.$_{AMPA}$ was significantly reduced from 60.0 ± 6.2 ($n=6$) to $14.8 \pm 3.1\%$ ($n=4$) (Figure 5b). These results indicate that a presynaptic mechanism may contribute to the anoxia-induced LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus.

Ca^{2+}-dependence of the anoxic LTP

Several lines of evidence have demonstrated that the induction and maintenance of the long-term enhancement of glutamatergic synaptic transmission in the CA1 region of the hippocampus requires a rise in intracellular Ca^{2+} concentration (Dudek & Bear, 1992; Mulkey & Malenka, 1992; Malenka & Nicoll, 1993), which may arise from an enhancement of external Ca^{2+} influx or an increase of release from intracelluar Ca^{2+} stores. To investigate the role of external Ca^{2+} on the anoxia-induced LTP of the e.p.s.p., we substituted Mg^{2+} for Ca^{2+} in the medium to abolish Ca^{2+}-influx. As shown in Figure 6, the initial slope of the e.p.s.p. was completely blocked by Ca^{2+}-free and anoxic ACSF. However, no persistent potentiation of synaptic transmission was observed on return to reoxygenated and Ca^{2+}-containing control ACSF solution in all 8 neurones tested. The mean initial slope of e.p.s.p. measured 30 min after the reoxygenation was $104.4 \pm 5.8\%$ ($P>0.1$, $n=8$). In another 6 experiments, we also found that the persistent potentiation of the initial slope of e.p.s.p. induced by the anoxic episode was completely abolished in a Ca^{2+}-free ACSF solution containing 5 mM EGTA (data not shown). These results indicate that the external Ca^{2+} is essential for the induction of the anoxic LTP.

Inhibition of the anoxia-induced LTP by Ca^{2+} chelator

In order to examine the role of intracellular Ca^{2+} concentration of the postsynaptic neurones in the anoxia-induced LTP of the e.p.s.p., we buffered the intracellular-free Ca^{2+} of the postsynaptic hippocampal CA1 neurones by intracellular application of the Ca^{2+} chelator BAPTA (500 mM) for at least 40 min before the anoxic episode. The effectiveness of BAPTA treatments was confirmed by the finding that the afterhyperpolarizations (AHPs) evoked by a depolarization current pulse injection (0.5–0.6 nA for 200 ms) was abolished. As illustra-

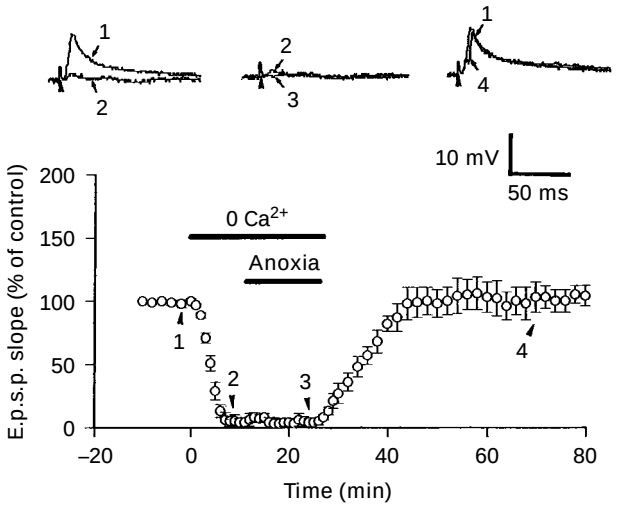

Figure 6 The anoxia-induced LTP of the initial slope of the e.p.s.p. was blocked by Ca^{2+}-free solution. The percentage change of the initial slope of the e.p.s.p. is plotted as a function of time. Bars denote the periods of delivery of Ca^{2+}-free solution and the anoxic episode. Upper traces are representative of those taken at different times as indicated.

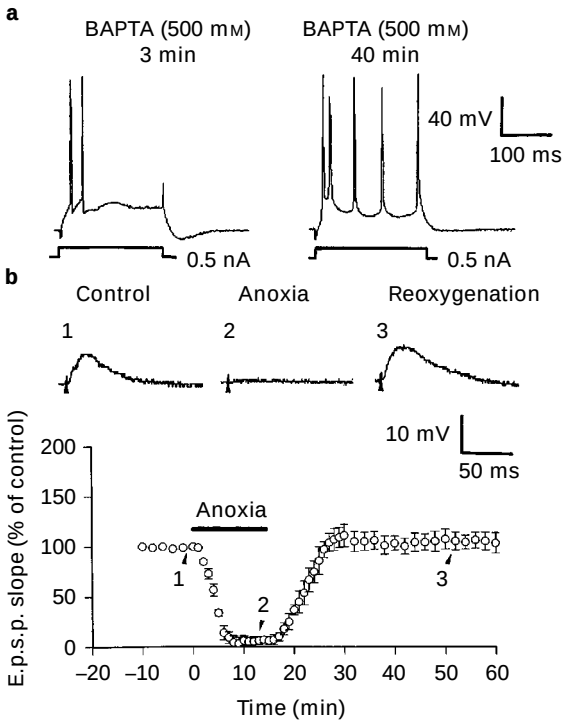

Figure 7 Intracellular application of the Ca^{2+} chelator, BAPTA, blocked the anoxia-induced LTP of the e.p.s.p. (a) Blockade of the AHP and spike frequency accommodation by intracellular application of BAPTA (500 μM), indicating the intracellular free Ca^{2+} has been successfully buffered by BAPTA loading (evoked at $V_m = -61$ mV). (b) The percentage change of the initial slope of the e.p.s.p. in the BAPTA-loaded cell was plotted against time. Note that the synaptic response was completely abolished during the period of the anoxic episode. On return to reoxygenated medium, the e.p.s.p. slope recovered rapidly to the control baseline. In all 8 BAPTA-loaded cells tested, no potentiation of the initial slope of the e.p.s.p. was observed on return to reoxygenated medium. Representative traces taken at different times as indicated. Bar denotes the period of delivery of the anoxic episode. Solid triangles represent the point of synaptic stimulation.

ted in Figure 7a, the response of a typical hippocampal CA1 neurone to depolarizing current pulses (0.5 nA for 200 ms) consisted of an initial rapid train of action potentials that slowed or accommodated. The train of action potentials was followed by an AHP. Intracellular application BAPTA (500 mM) for 40 min blocked the AHP and spike frequency accommodation was reduced, indicating that this cell sucessfully buffered the intracellular free Ca^{2+} by BAPTA loading. As shown in Figure 7b, the initial slope of the e.p.s.p. was fully blocked (mean maximal depression in 8 neurones was $94.2 \pm 4.3\%$) when the hippocampal slices were introduced into the anoxic ACSF medium within 10–15 min. On return to reoxygenated medium, the initial slope of the e.p.s.p. progressively returned to the control values within 10–15 min. In all 8 BAPTA-loaded neurones tested, no potentiation of e.p.s.p. slope was observed on return to reoxygenated medium. The mean initial slope of the e.p.s.p. of the BAPTA-loaded cells measured 30 min after the reoxygenation was $103.8 \pm 6.9\%$ ($P > 0.1$, $n = 8$). These results indicate that the rise of intracellular free Ca^{2+} is essential for the generation of the anoxic LTP in the CA1 region of rat hippocampus.

The relationship between the anoxia-induced LTP and the tetanic LTP of e.p.s.ps

Because the persistence of the anoxia-induced long-term potentiation of glutamatergic synaptic transmission is similar to that observed during LTP induced by high-frequency stimulation (tetanic LTP), we examined whether these two forms of synaptic potentiation could occlude each other, as an indication that they share a common cellular mechanism. We first determined the effect of the saturated tetanic LTP, which was given by three episodes of tetanic stimulation repeated every 20 min, on the induction of the anoxic LTP. As shown in Figure 8, the first application of the tetanic stimulation (100 Hz for 1 s) induced LTP that stabilized at a value of approximately $221.4 \pm 18.3\%$ ($n = 8$) of the pre-conditioning baseline response. However, a third episode of conditioning stimulation had no additional effect, indicating that the tetanic LTP was saturated. The stimulus intensity was then adjusted such that the initial slope of the e.p.s.p. matched pre-tetanus level, and a long-period of the anoxic episode (15 min) was delivered. As shown in Figure 8, the saturated tetanic LTP significantly occluded the anoxia-induced LTP, but the depression of the e.p.s.p. slope during the anoxic episode was not affected by the prior three trains of tetanic stimulation. The mean initial slope of the e.p.s.p. measured 60 min following the 15 min of anoxic episode was $105.4 \pm 5.8\%$ ($n = 8$). We next examined whether the anoxic LTP could occlude the tetanic LTP induced by a high-frequency tetanic stimulation. As illustrated in Figure 9, the anoxia-induced LTP was induced by a long-period (15 min) of the anoxic episode and stabilized for 20 min. The stimulus intensity was then reduced to generate the initial slope of the e.p.s.p. to the pre-anoxic level. After a stable baseline had been obtained for 5 min, a high frequency tetanic train (100 Hz for 1 s duration) was delivered and the synaptic response to the tetanic stimulation was measured for more than 1 h. We found that the prior anoxia-induced potentiation of synaptic transmission significantly attenuated the expression of tetanic LTP induced by a high-frequency tetanic stimulation (Figure 9). In all 8 hippocampal slices examined, the mean initial slope of the e.p.s.p. 60 min following the high-frequency stimulus was $110.6 \pm 10.2\%$ ($n = 8$, $P > 0.05$). Based on these findings, we suggest that the induction and expression of the tetanic LTP and the anoxic LTP in the CA1 region of rat hippocampus may share at least partially a common cellular pathway. Furthermore, the anoxic LTP was also observed as usual in three slices that were not stimulated for 10 min before nor during the application of the anoxic episode (the initial slope of the e.p.s.p. increased by $76.4 \pm 9.3\%$, $n = 3$, $P < 0.01$; data not shown). These results imply that the anoxic LTP does not depend on stimulation and is not pathway specific.

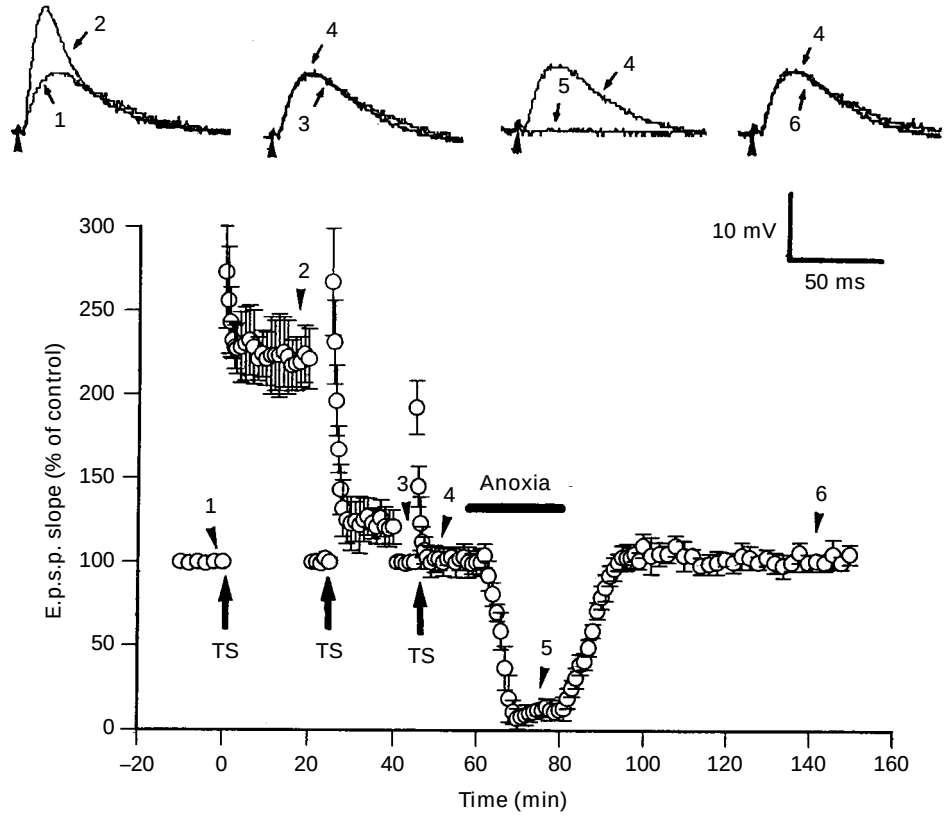

Figure 8 The LTP induced by tetanization occluded the anoxia-induced LTP of the e.p.s.p. Plot of the experiments in which tetanic stimulations (100 Hz for 1 s duration) were applied repetitively to saturate LTP. When the LTP became stable, the initial slope of the e.p.s.p. was decreased by reducing the stimulus intensity. Afer the LTP was saturated, a long-period of the anoxic episode (15 min) was delivered (evoked at $V_m = -60$ mV). At this time, the anoxic episode failed to induce a persistent potentiation of the e.p.s.p. slope ($n = 8$). Representative traces taken at different times as indicated. Bar denotes the period of delivery of the anoxic episode. Solid triangles represent the point of synaptic stimulation. TS denotes the point of tetanic stimulation.

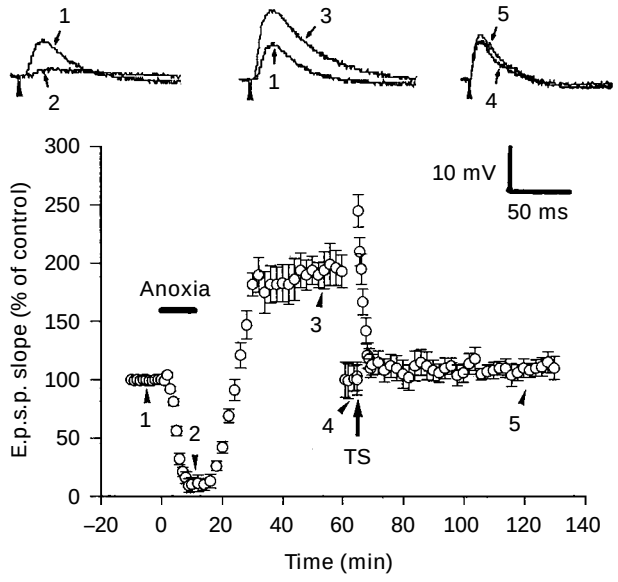

Figure 9 The anoxic LTP attenuated the expression of the tetanic LTP induced by tetanization. The anoxic LTP was induced by a long-period of the anoxic episode (15 min duration) and stabilized for 20 min (evoked at $V_m = -61$ mV). After a stable baseline had been obtained, the stimulus intensity was reduced until the initial slope of the e.p.s.p. was at the pre-anoxic level. This treatment significantly attenuated the subsequent potentiation of synaptic plasticity induced by a high-frequency tetanic stimulation (100 Hz for 1 s duration) ($n = 8$). Representative traces taken at different times as indicated. Bar denotes the period of delivery of the anoxic episode. Solid triangles represent the point of synaptic stimulation. TS denotes the point of tetanic stimulation.

Discussion

There are three main findings of the present study. First, the level of persistent potentiation of the AMPA and NMDA receptor-mediated components of synaptic transmission induced by the anoxic episodes was similar, both in terms of amplitude and time course. Additionally, both a persistent enhancement of glutamate release from presynaptic nerve terminals and a persistent enhancement of the postsynaptic NMDA receptor-mediated synaptic response are responsible for the generation of the anoxic LTP. Second, we have found that the anoxia-induced LTP of the initial slope of the e.p.s.p. was dependent on the external Ca^{2+}. In addition, intracellular injection of the hippocampal CA1 neurones with the Ca^{2+} chelator BAPTA prevented the induction of the anoxic LTP. Third, the saturated tetanic LTP evoked by three episodes of tetanic stimulation could effectively occlude the induction of the anoxic LTP. Conversely, prior anoxia-induced potentiation of synaptic transmission also significantly attenuated the subsequent induction of the tetanic LTP.

Both AMPA and NMDA receptors contribute to the anoxia-induced LTP of e.p.s.ps

The results of the present study provide strong evidence that the anoxia-induced LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus is associated with enhancement of both AMPA and NMDA receptor-mediated components of synaptic transmission. The anoxic episodes induce a persistent potentiation of the initial slopes of both e.p.s.p.$_{AMPA}$ and e.p.s.p.$_{NMDA}$ with a similar time course and magnitude to that observed for the composite e.p.s.p. These results are not consistent with those of Crépel *et al.* (1993), who showed that the induction and maintenance of a short

(2–3 min) anoxic-aglycaemic episode- induced LTP are only due to a persistent upregulation of the NMDA receptor-mediated component of the synaptic transmission without involvement of the AMPA receptor-mediated component. The difference between the Crépel's findings and our present results may be due to the difference in the severity of the insult (anoxia-aglycaemia vs anoxia) or the duration of the insult (2–3 min vs 15 min). Crépel *et al*. (1993) found that the anoxic-aglycaemic LTP could be blocked by the NMDA receptor antagonist DL-APV. However, they did not examine directly whether the pharmacologically isolated AMPA receptor-mediated synaptic transmission might also be persistently potentiated and have therefore not excluded this possibility.

Both presynaptic and postsynaptic mechanisms contributed to the anoxia-induced LTP of e.p.s.ps

An important finding of the present study was that a presynaptic mechanism seems to be involved in the persistent potentiation of glutamatergic synaptic transmission induced by the anoxic episodes. Evidence supporting this is that the persistent potentiation of $e.p.s.p._{AMPA}$ and $e.p.s.p._{NMDA}$ had a similar time course and magnitude (Figures 2 and 3). Equal changes in $e.p.s.p._{AMPA}$ and $e.p.s.p._{NMDA}$ would be expected to arise from a presynaptic mechanism in which presynaptic neurotransmitter release mechanisms are altered. Furthermore, in agreement with this conclusion, we have also found that the persistent potentiation of the initial slope of $e.p.s.p._{AMPA}$ following the anoxia episodes was accompanied by a decrease in PPF ratio, a phenomenon generally accepted to reflect presynaptic changes (Figure 5). It was therefore concluded that a presynaptic mechanism may be involved in the anoxia-induced LTP. Interestingly, a similar rebound of sustained potentiation of excitatory synaptic transmission has also been shown following a temporary suppression of glycolysis in slices of the rat hippocampus *in vitro*. Because this form of long-lasting enhancement of synaptic transmission was generated by substituting 2-deoxy-D-glucose (2-DG) for glucose to suppress the glycolysis at the hexokinase step, it was termed 2-DT-LTP (Tekkök & Krnjevic, 1995). On comparing the properties of anoxic LTP with those of 2-DG-LTP, we have found that the mechanisms leading to the induction of these two forms of LTP share several similarities. For example, both require an increase in presynaptic release and are not pathway-specific. However, despite these similarities, there are major differences. For example, unlike anoxic LTP, 2-DG-LTP is not occluded by a preceding tetanic LTP and is insensitive to block of nitric oxide (NO) synthesis (Huang & Hsu, 1997).

However, the present results do not exclude the possibility that the postsynaptic modulation of the glutamatergic receptor-mediated synaptic response is also involved in the anoxia-induced LTP of synaptic transmission. The postsynaptic responses induced by exogenous application of NMDA were persistently potentiated following the anoxic episode, indicating that the generation of the anoxic LTP might require a persistent upregulation of the postsynaptic NMDA receptor-mediated synaptic response. This result is in agreement with the previous finding of Crépel *et al*. (1993), who showed that the NMDA receptor-mediated currents on the rat hippocampal CA1 pyramidal neurone were persistently potentiated following a short period of anoxia-aglycaemia. Similarly, Gozlan *et al*. (1994) also provided strong evidence that the redox site of the NMDA receptors is involved in the induction and the maintenance of the anoxia-aglycaemia-induced LTP. They showed that thiol oxidizing compound such as 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) could effectively inhibit this anoxic LTP. Taken together, these results seem to favour the idea that the postsynaptic upregulation of the NMDA receptor-mediated synaptic response is possibly involved in the generation of the anoxia-induced LTP of glutamatergic synaptic transmission in the CA1 region of the rat hippocampus.

An interesting question derived from our present work is why the anoxic episode selectively potentiates the postsynaptic NMDA receptor-mediated response but not the postsynaptic AMPA receptor-mediated response. Since it has been shown that the anoxic episode can increase reducing activity, it will enhance NMDA receptor-mediated response via the redox sites of the receptor-channel complex (Gozlan *et al*., 1994). Thus, one possibility to explain the lack of effect of the anoxic episode on the postsynaptic AMPA receptor-mediated response is that the AMPA receptor lacks the redox sites, present on the NMDA receptor-channel complex, which can be modulated by the biochemical cascades activated by the anoxia.

The role of Ca^{2+} in the anoxia-induced LTP of e.p.s.ps

It has been shown that the influx of Ca^{2+} into the postsynaptic pyramidal cells and the resulting rise in the intracellular Ca^{2+} concentration are critical requirements for the induction of multiple forms of activity-dependent plasticity (e.g. high-frequency tetanus stimulation-induced long-term potentiation and long-term depression) occurring at the Schaffer collateral-commissural CA1 synapses of the hippocampus (Malenka, 1991; Brocher *et al*., 1992). In the present study, we found that the persistent potentiation of the e.p.s.p. following the anoxic episode was abolished when synaptic transmission was blocked by removal of external Ca^{2+} (Figure 6), suggesting that the enhancement of Ca^{2+} influx into presynaptic nerve terminal or postsynaptic pyramidal neurones is required for the generation of the anoxic LTP in CA1.

It is generally believed that the maintenance of a long-term modification of synaptic plasticity in the CA1 region of rat hippocampus may require a retrograde messenger that is released from the postsynaptic pyramidal neurones and diffuses across the synaptic cleft to modify presynaptic function (Malenka & Nicoll, 1993; Zhuo *et al*., 1994). Recently, a number of possible substances, such as nitric oxide, carbon monoxide and arachidonic acid, has been show to act as retrograde messengers for the maintenance of the tetanic LTP in the hippocampus (Malenka & Nicoll, 1993; Zhuo *et al*., 1994). In our recent study, we have provided strong evidence that blockade of nitric oxide synthase (NOS) during the anoxic episode prevents the generation of the anoxic LTP in the CA1 (Huang & Hsu, 1997). These results indicate that NO is involved in the generation of the anoxic LTP by inducing the presynaptic nerve terminals to enhance glutamate release. The present study indirectly supports this hypothesis, demonstrating that the induction of anoxic LTP was prevented by intracellular injection of the Ca^{2+} chelator BAPTA (500 mM) into the postsynaptic CA1 neurones (Figure 7). These results are consistent with those of Crépel and Ben-Ari (1996), who also demonstrated that intracellular injection of BAPTA could effectively prevent the generation of anoxic LTP induced by a short period (2–3 min) of the anoxic-aglycaemic episodes. These findings indicated that the intracellular free Ca^{2+} of the postsynaptic neurones is essentially required for the induction of the anoxic LTP. Because the release of retrograde messengers from the postsynaptic neurones is dependent on the intracellular free Ca^{2+}, reducing intracellular free Ca^{2+} could be expected to reduce the release of retrograde messengers and then fail to maintain the long-term enhancement of synaptic plasticity that follows certain types of tetanic stimulation of glutamatergic afferents (Bliss & Collingridge, 1993; Hawkins *et al*., 1993). Therefore, it is possible that the expression of anoxic LTP is related to the retrograde messenger(s) released from postsynaptic neurones which then enhance glutamate release from the presynaptic terminals.

The interactions between tetanic LTP and anoxia-induced LTP of e.p.s.ps

Tetanic LTP is a model of synaptic plasticity that is thought to contribute to certain forms of learning and memory (Malenka & Nicoll, 1993; McNaughton, 1993). In physiological conditions, the induction and maintenance of tetanic LTP in CA1

are mediated by NMDA and AMPA receptors, respectively (Malenka & Nicoll, 1993; McNaughton, 1993). Evidence has been obtained that the induction of the tetanic LTP requires Ca^{2+} influx through postsynaptic NMDA receptor-channel complex, which in turn triggers a cascade of events leading to the expression of the LTP. The maintenance of the LTP apparently involves, at least in part, a presynaptic increase of glutamate release from nerve terminals (Malenka & Nicoll, 1993; Zhuo *et al*., 1994). If tetanic- and anoxia-induced LTP share similar molecular pathway(s), they should reciprocally occlude one another. We have observed that saturated tetanic LTP could effectively occlude the subsequent induction of the anoxic LTP (Figure 8). Conversely, prior induction of the anoxic LTP significantly attenuated subsequent tetanic LTP (Figure 9). These findings strongly suggest that these two forms of synaptic enhancement share a common cellular mechanism in their induction. The idea of a common mechanism in the induction of tetanic and anoxic LTP is also advocated by the following observations. It has been found that (1) both are prevented by postsynaptic injection of BAPTA (Hammond *et al*., 1994); and (2) both are prevented by NOS inhibitors, suggesting that NO-mediated biochemical cascades are important factors in their induction mechanism (Malenka & Nicoll, 1993; Zhuo *et al*., 1994; Huang & Hsu, 1997). However, despite these similarities, there is a prominent difference between the induction of tetanic and anoxic LTP. Unlike tetanic LTP, anoxic LTP is insensitive to block of the NMDA receptor. Because the results of this and a previous study suggest that the induction of anoxic LTP requires Ca^{2+} influx (Crépel & Ben-Ari, 1996), an issue that must be considered is how Ca^{2+} enters to trigger the anoxic LTP. Recently, it has been widely accepted that Ca^{2+} influx through voltage-dependent Ca^{2+} channels is also involved in the induction of some types of LTP in the hippocampal CA1 region (e.g. TEA-induced LTP) (Huang & Malenka, 1993). One possible interpretation of this finding is that voltage-dependent Ca^{2+} channels may permit substantial Ca^{2+} influx, triggering AMPA receptor-mediated anoxic LTP when the NMDA receptor-mediated synaptic response was blocked. Further experiments are required to test this possibility. Besides the difference in the induction processes, there is also a major difference between the expression of tetanic and anoxic LTP. The expression of tetanic LTP is mediated in physiological conditions primarily by AMPA receptors, whereas anoxic LTP can still be expressed following the blockade of the AMPA receptor-mediated synaptic response. These results indicate a possibility that these two forms of synaptic enhancement use at least one independent cellular mechanism in their maintenance processes. A similar result has been obtained in the rat hippocampal CA1 region following transient anoxic-aglycaemic episodes (Crépel & Ben-Ari, 1993; Hammond *et al*., 1994). Consistent with these *in vitro* findings, the *in vivo* animal studies have shown that rats exposed to a neonatal anoxic insult exhibited a persistent disturbance of spatial learning and memory into adult life (Dell'Anna *et al*., 1991; Iuvone *et al*., 1996). Taking published data and our own together, we suggest that the anoxic episodes could not only induce the neuronal damage, but also impair the processes of learning and memory in the central nervous system.

In conclusion, based on the findings of the present study and our recent work (Huang & Hsu, 1997), we propose that the following mechanisms possibly underlie the anoxic LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus. The anoxic episodes induce a persistent depolarization of the postsynaptic pyramidal cells which in turn promotes the entry of Ca^{2+}, and then one or more diffusable retrograde messengers (e.g. NO) is (are) released from the postsynaptic dendrites and diffuse(s) back to the presynaptic nerve terminals to enhance glutamate release. In addition, the upregulation of the postsynaptic NMDA receptor-mediated synaptic response might be also involved in the generation of the anoxic LTP. Because several observations suggest that the anoxic LTP of glutamatergic synaptic transmission in the CA1 region of rat hippocampus plays an important role in the delayed hippocampal CA1 neuronal death triggered by a global ischaemic episode (Crépel *et al*., 1993; Hammond *et al*., 1994), our findings may shed light on the precise mechanisms of the delayed onset of the neuronal death which is induced by anoxic or ischaemic insults *in vivo*.

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