Glycolysis Prevents Anoxia-Induced Synaptic Transmission Damage in Rat Hippocampal Slices

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Tian, Guo-Feng and Andrew J. Baker. Glycolysis prevents anoxia-induced synaptic transmission damage in rat hippocampal slices. *J. Neurophysiol.* 83: 1830–1839, 2000. Prolonged anoxia can cause permanent damage to synaptic transmission in the mammalian CNS. We tested the hypothesis that lack of glucose is the major cause of irreversible anoxic transmission damage, and that anoxic synaptic transmission damage could be prevented by glycolysis in rat hippocampal slices. The evoked population spike (PS) was extracellularly recorded in the CA1 pyramidal cell layer after stimulation of the Schaffer collaterals. When the slice was superfused with artificial cerebrospinal fluid (ACSF) containing 4 mM glucose, following 10 min anoxia, the evoked PS did not recover at all after 60 min reoxygenation. When superfusion ACSF contained 10 mM glucose with or without 0.5 mM α-cyano-4-hydroxycinnate (4-CIN), after 60 min reoxygenation the evoked PS completely recovered following 10 min anoxia. When superfusion ACSF contained 20 mM glucose with or without 1 mM sodium cyanide (NaCN), after 60 min reoxygenation the evoked PS completely recovered even following 120 min anoxia. In contrast, when superfusion ACSF contained 4 mM glucose, following 10 min 1 mM NaCN chemical anoxia alone, without anoxic anoxia, the evoked PS displayed no recovery after 60 min reoxygenation. Moreover, when 16 mM mannitol and 16 sodium L-lactate were added into 4 mM glucose ACSF, following 10 min anoxia the evoked PS failed to recover at all after 60 min reoxygenation. The results indicate that elevated glucose concentration powerfully protected the synaptic transmission against anoxic damage, and the powerful protection is due to anaerobic metabolism of glucose and not a result of the higher osmolality in higher glucose ACSF. We conclude that lack of glucose is the major cause of anoxia-induced synaptic transmission damage, and that if sufficient glucose is supplied, glycolysis could prevent this damage in vitro.

INTRODUCTION

Neurons in the mammalian CNS may be functionally impaired following anoxic exposure (Fujiwara et al. 1987; Kass and Lipton 1982, 1986, 1989; Krnjević and Leblond 1989; Lipton and Whittingham 1979). Anoxia for 10–15 min in vivo causes irreversible morphological (Pulsinelli et al. 1982; Von Lubitz and Diemer 1983) and functional (Keykham et al. 1978; Smith et al. 1983) damage to brain tissue. Anoxia for 10 min in vitro also leads to irreversible loss of synaptic transmission (Kass and Lipton 1982, 1986, 1989; Lipton and Whittingham 1979).

Because little glycogen is stored in brain tissue, a continuous supply of glucose is crucial for neuronal function in the mammalian CNS (Barinaga 1997; Nelson et al. 1968). Although many investigators have examined the changes in the intrinsic membrane properties during anoxia (Belousov et al. 1995; Fried et al. 1995; Hershkowitz et al. 1993; Jiang and Haddad 1994; Krnjević and Leblond 1989; Zhang and Krnjević 1993) and protection against anoxic damage (Clark and Rothman 1987; Fried et al. 1995; Schurr et al. 1995), few papers have addressed the issue of the energy supply during anoxia (Grigg and Anderson 1989; Lipton and Whittingham 1982; Schurr et al. 1987; Zhang and Krnjević 1993) even though glucose deprivation causes transient or permanent loss of neuronal functions (Fowler 1993; Shoji 1992; Wada et al. 1997).

There are discrepant conclusions arising from the results of studies on either mechanisms or the dose response of anoxic injury in brain slice preparations. For example, after 10 min anoxia there was almost no recovery of CA1 synaptic transmission in slices from young and adult rats (Kass and Lipton 1989); in contrast, after up to 20 min anoxia, synaptic transmission almost completely recovered (Grigg and Anderson 1989). We suggest that the concentration of glucose in the artificial cerebrospinal fluid (ACSF) is a critical factor. The concentration of glucose in ACSF varied from 4 mM (Fried et al. 1995; Kass and Lipton 1982, 1986, 1989; Lipton and Whittingham 1979, 1982), 7 mM (Clark and Rothman 1987), 10 mM (Belousov et al. 1995; Hershkowitz et al. 1993; Jiang and Haddad 1994; Krnjević and Leblond 1989; Schurr et al. 1987; Zhang and Krnjević 1993), 11 mM (Fujiwara et al. 1987; Grigg and Anderson 1989; Shoji 1992), and up to 25 mM (Jiang and Haddad 1994). This has usually been neglected in discussions of discrepant experimental results of in vitro studies (i.e., Fujiwara et al. 1987; Jiang and Haddad 1994; Kass and Lipton 1989). In this study, we demonstrate that variations in glucose concentrations may account for the differences in results (Grigg and Anderson 1989; Kass and Lipton 1989) and that glucose powerfully protects synaptic transmission against anoxia-induced damage in vitro.

Although some investigators (Grigg and Anderson 1989; Schurr et al. 1987) have noted that increased glucose concentrations in ACSF improved recovery of neuronal function from anoxic challenge, the duration of anoxia has never exceeded 60 min in brain slices. Our results show that superfusion of hippocampal slices with ACSF containing sufficient glucose sustains partial synaptic transmission during anoxia and ensures its rapid recovery after hypoxia is terminated. These results demonstrated that during anoxia, lack of glucose impairs synaptic transmission. If sufficient glucose is supplied

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during anoxia, synaptic transmission is partially supported and completely protected by glycolysis.

METHODS

Male Sprague-Dawley rats (150–200 g) were anesthetized with 2.0–2.5% halothane in oxygen (Tian et al. 1999) and decapitated. Hippocampal slices from rat brains were prepared as described previously (Zhang et al. 1998). The brain was immediately removed and maintained in an ice-cold ACSF for 3–5 min before slicing. The brain was mounted on an aluminum block and transversely sliced (~400 μm) in ice-cold (<3°C) ACSF using a vibratome (series 1000, Technical Products International, St. Louis, MO). Slices then were kept in oxygenated ACSF at room temperature (22–23°C) for at least 1 h before the experiment and not more than 8 h after slice preparation. The composition of the ACSF is (in mM) 126 NaCl, 3.0 KCl, 1.4 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaCO_3$, and 4 glucose (Kass and Lipton 1989), pH 7.4 at 36.5 ± 0.5°C when aerated with 95% O_2-5% CO_2.

For electrophysiological recording, the slice was placed in a superfusion chamber, which was closed up like a box by adding removable plates, and only a small slit remained, which gave access to the electrodes. The slice was fully submerged in the superfusion chamber and continuously superfused (7–8 ml/min) with ACSF equilibrated and continuously bubbled with 95% O_2-5% CO_2 (Zhang et al. 1998). Humidified, warmed 95% O_2-5% CO_2 was blown over the chamber to ensure a warm oxygenated local environment (Zhang et al. 1998). All recordings were made at slice temperature of 36–37°C. To achieve a stable experimental temperature, the ACSF was warmed up before it superfused the slice using a water bath controlled by a temperature controller, and the temperature of the ACSF in the superfusion chamber was continuously monitored using a YSI 4600 series precision thermometer with a micro YSI 451 temperature sensor (YSI, Yellow Springs, OH). The slice was made "anoxic" by superfusing them with ACSF preequilibrated and continuously bubbled with 95% N_2-5% CO_2, and humidified, warmed 95% N_2-5% CO_2 was blown over the chamber to ensure a warm oxygen-free local environment. Field potentials were recorded extracellularly through glass pipettes filled with 150 mM NaCl (tip resistance of 2–3 MΩ), and the electrode was placed in the somatic layer of the CA1 region. Signals were recorded using an Axopatch 200B amplifier (Axon Instrument, Foster City, CA), and data were stored and analyzed with pCLAMP software (version 6.0.4, Axon Instrument).

Electrical stimulation of Schaffer collaterals was performed using a bipolar tungsten electrode placed in the stratum radiatum of CA1. Stimulation pulses of constant current (0.2–0.5 mA, 0.1 ms) were generated by a Grass S88 stimulator (Grass Instrument, West Warwick, RI) and delivered through an isolation unit (PSIU6) every 30 s.

The extracellular postsynaptic orthodromic responses started with a small downward stroke (arrows in Fig. 1*A*) just after the stimulus artifact, the presynaptic volley (PV) (Fried et al. 1995). The PV was followed by an upward waveform [field excitatory postsynaptic potential (fEPSP)]. During the fEPSP, there was a sharp downward stroke, the postsynaptic population spike (PS; asterisk in Fig. 1*A*) (Fried et al. 1995). The PS amplitude was evaluated by calculating the voltage difference between the negative peak and the positive one preceding it.

To examine the effect of glucose on synaptic transmission during and after anoxia, slices were superfused with ACSF that contained different concentrations of glucose. In one set of experiments, glucose concentrations were 4, 10, and 20 mM. In another series, glucose was used in combination with other chemicals [4 mM glucose plus 16 mM mannitol, 4 mM glucose plus 16 mM sodium L-lactate, 4 mM glucose plus 1 mM sodium cyanide (NaCN), 10 mM glucose plus 0.5 mM α-cyano-4-hydroxycinnate (4-CIN), 20 mM glucose plus 1 mM NaCN]. Different ACSFs were superfused for 15 min before anoxia, during anoxia, and 60 min reoxygenation after different durations (10, 60, and 120 min) of anoxia, except ACSF containing 4 or 20 mM glucose plus 1 mM NaCN, which were superfused only during anoxia. In this case ACSF containing 4 or 20 mM glucose without NaCN was superfused for 15 min before anoxia and during 60 min reoxygenation. Recovery of synaptic transmission was assessed by expressing the 1-h postanoxic PS amplitude as a percentage of control (preanoxic) amplitude.

All data are expressed as group means ± SD. Significance levels in all cases were determined by Student's *t*-test.

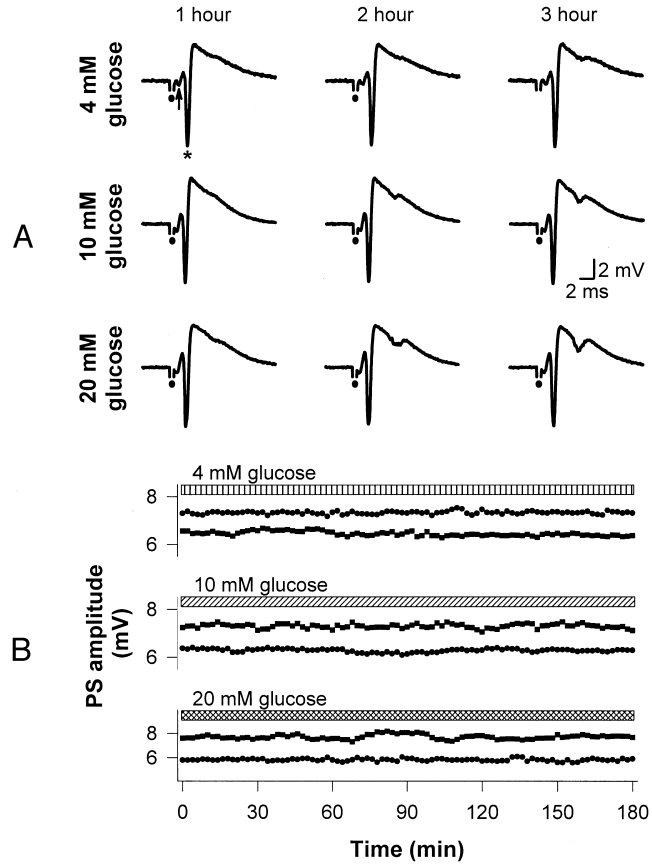

FIG. 1. Extracellular recordings were kept stable for up to 3 h in artificial cerebrospinal fluid (ACSF) without anoxic challenges. *A*: recordings were obtained from the CA1 pyramidal cell layer after stimulation of the Schaffer collaterals at 1, 2, and 3 h in ACSF containing 4, 10, and 20 mM glucose. ●, stimulation artifacts; ↑, presynaptic volley (PV), representing action potentials from the axonal terminals of the Schaffer collaterals; *, population spike (PS), consisting of the synchronized firings of postsynaptic neurons. All recordings are shown in the same scales. *B*: plots of PS amplitude vs. time when the slice was superfused with ACSF containing 4, 10, and 20 mM glucose (2 data obtained from each glucose concentration in ACSF), indicating that recordings were kept stable for up to 3 h.

RESULTS

Extracellular recordings of evoked responses

Figure 1*A* shows the synaptic responses recorded from the CA1 pyramidal cell layer after electrical stimulation of the Schaffer collaterals when the slice was superfused with ACSF containing 4, 10, and 20 mM glucose. The responses were similar to those reported previously (Fried et al. 1995). The typical response consists of three parts: the PV, the fEPSP, and the PS. The PV is a small downward stoke (arrow in Fig. 1*A*) just after the stimulus artifacts (Fried et al. 1995), representing

action potentials from the axonal terminals of the Schaffer collaterals. The fEPSP is an upward waveform just after the PV. The PS is a sharp downward stroke during the fEPSP (Fried et al. 1995), consisting of the synchronized firings of postsynaptic neurons, its magnitude largely determined by the number and synchronization of their firings (asterisk in Fig. 1*A*). The extracellular recordings were stable for up to 3 h when the slice was superfused with ACSF containing 4, 10, and 20 mM glucose in normal conditions without any anoxic challenge (Fig. 1*B*). The maximal PS amplitudes averaged 7.1 ± 1.3 mV (mean ± SD, range 5.0–13.0 mV, $n = 86$).

Effects of anoxia on evoked responses

When the slice was superfused with ACSF containing 4 mM glucose, the PV was abolished within 5 min after introduction of anoxia ($n = 6$; Fig. 2*A*). However, it persisted throughout 10 min ($n = 6$; Fig. 3*A*) and 60 min ($n = 7$; Fig. 3*C*) of anoxia at 10 mM glucose; it also persisted throughout 60 min ($n = 6$; Fig. 4*A*) and 120 min ($n = 6$; Fig. 4*C*) of anoxia at 20 mM glucose. Thus severe prolonged anoxia did not impair the ability of Schaffer collaterals to be excited and conduct action

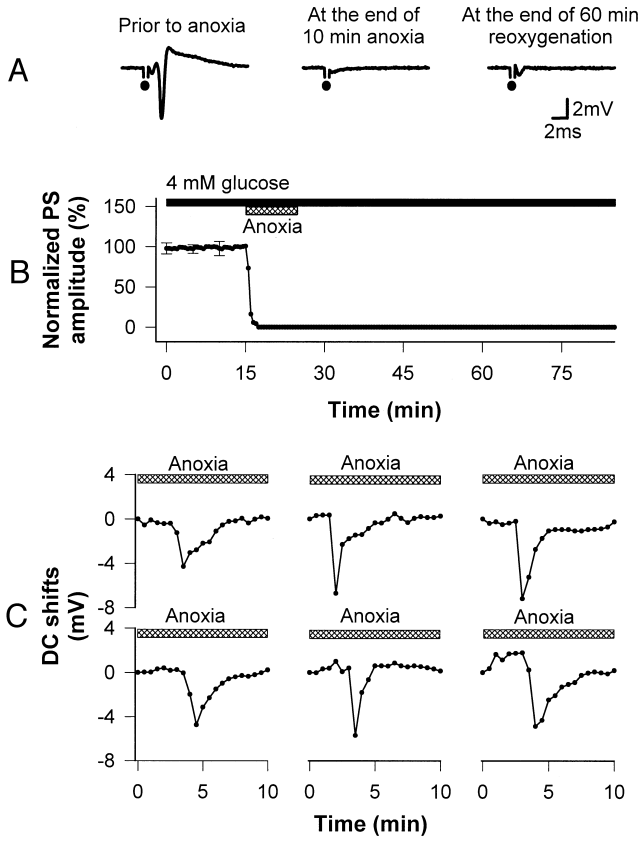

FIG. 2. Recovery of synaptic transmission after 10 min anoxia at 4 mM glucose in ACSF. *A*: recordings were obtained before anoxia, at the end of 10 min anoxia and at the end of 60 min reoxygenation. ●, stimulation artifacts. *B*: normalized PS amplitudes were obtained during 15 min before anoxia, during 10 min anoxia, and during 60 min reoxygenation. The PS did not recover, indicating irreversible damage to synaptic transmission by 10 min anoxia at 4 mM glucose in ACSF. Error bars represent SD (5-min intervals). *C*: DC shifts were obtained during 10 min anoxia in 6 slices (30-s intervals). Anoxic depolarization occurred within 2–5 min after the introduction of anoxia in every recording at 4 mM glucose in ACSF, indicating the ion gradients across neuronal membrane were severely disturbed.

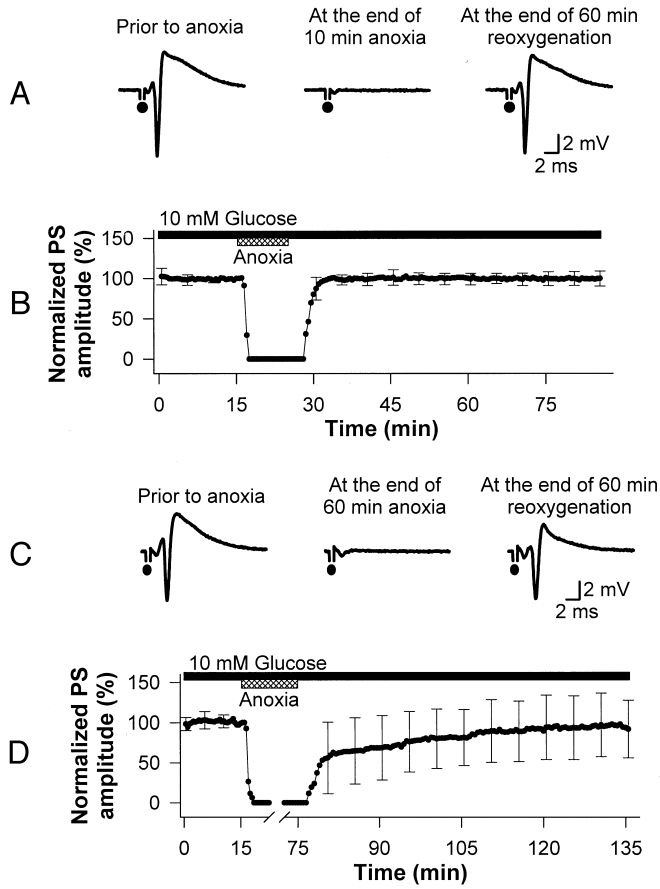

FIG. 3. Recovery of synaptic transmission after 10 min anoxia (*A* and *B*) or 60 min anoxia (*C* and *D*) at 10 mM glucose in ACSF. Recordings were obtained before anoxia, at the end of 10 min anoxia (*A*) or 60 min anoxia (*C*) and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A* and *C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during 10 min anoxia (*B*) or 60 min anoxia (*D*), and during 60 min reoxygenation in *B* and *D*. The PS completely recovered after 10 min anoxia or partially recovered after 60 min anoxia at 10 mM glucose in ACSF, indicating that glucose at 10 mM in ACSF protected synaptic transmission against damage by 10 min anoxia and even 60 min anoxia. Error bars represent SD (5-min intervals).

potentials when the slices were superfused with ACSF containing 10 or 20 mM glucose.

At 4 mM glucose, the fEPSP in all slices completely disappeared at ~2–4.5 min after introduction of anoxia (Table 1). At 10 mM glucose, however, the fEPSP in some slices did not disappear during 10 min (2 of 6) and 60 min (2 of 7) anoxia at all (Table 1); the fEPSP in some slices disappeared at ~3–8 min after introduction of anoxia, but the fEPSP in some slices recovered later during anoxia (Table 1). Similar to those at 10 mM glucose, at 20 mM glucose, and during 60 and 120 min anoxia, the fEPSP in some of slices did not disappear at all throughout the duration of anoxia (Fig. 5*A*, Table 1); the fEPSP in some slices disappeared ~4–10 min after introduction of anoxia, but the fEPSP in some slices recovered later during anoxia (Fig. 5*B*, Table 1). The retention of the fEPSP in some slices (Fig. 5*A*) and its reappearance in others (Fig. 5*B*) during anoxia at 10 or 20 mM glucose, particularly at 20 mM glucose during anoxia lasting 120 min, indicated that presynaptic terminals could still be excited and could retain the ability to release neurotransmitters, and that postsynaptic membrane re-

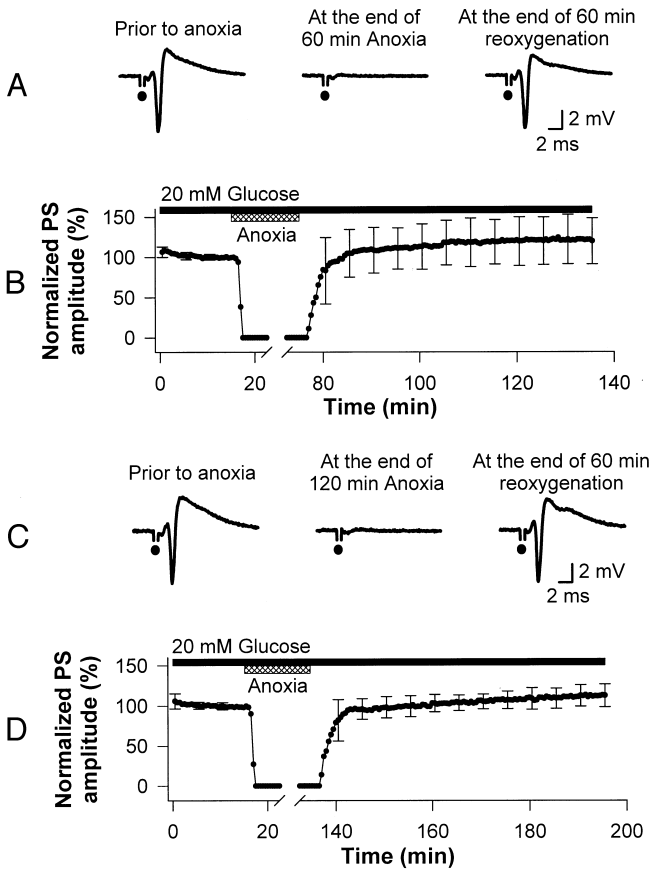

FIG. 4. Recovery of synaptic transmission after 60 min anoxia (*A* and *B*) or 120 min anoxia (*C* and *D*) at 20 mM glucose in ACSF. Recordings were obtained before anoxia, at the end of 60 min anoxia (*A*) or 120 min anoxia (*C*), and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A–C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during 60 min anoxia (*B*) or 120 min anoxia (*D*), and during 60 min reoxygenation in *B* and *D*. PS was fully restored after 60 min anoxia and even 120 min anoxia at 20 mM glucose in ACSF, indicating that glucose at 20 mM in ACSF virtually prevented synaptic transmission against damage by 60 min anoxia and even 120 min anoxia. Error bars represent SD (5-min intervals).

ceptors retained the ability to respond to released neurotransmitters.

The PS was always depressed at ~0.5–1.5 min and completely abolished within 3 min after the introduction of anoxia regardless of glucose concentration ($P > 0.05$; Figs. 2–4).

At 4 mM glucose, the anoxic depolarization (Roberts and Sick 1988) always occurred within 2–5 min (3.3 ± 0.8 min, $n = 6$) after introduction of anoxia (Fig. 2*C*). The occurrence of anoxic depolarization indicated that the ion gradients across neuronal membrane were severely disturbed. However, at 10 or 20 mM glucose, anoxic depolarization was not observed during 10, 60, and even 120 min anoxia. Anoxic depolarization was not observed during 10, 60, and even 120 min of anoxia, suggesting that the extracellular potassium concentration was well maintained in ACSF containing 10 or 20 mM glucose.

TABLE 1. *Effect of anoxia on fEPSP at different glucose concentration in ACSF*

Glucose, mM	Anoxia, min	fEPSP Sustained*	fEPSP Abolished		
			Time, min†	*n*	Recovery‡
4	10	0	3.3 ± 0.8	6	0
10	10	2	6.0 ± 1.2	4	0
10	60	2	5.4 ± 1.8	5	2
20	60	2	5.1 ± 1.3	4	2
20	120	1	7.5 ± 2.5	5	4

Values in Time are means ± SD; *n* is number of slices. fEPSP, field excitatory postsynaptic; ACSF, artificial cerebrospinal fluid. * Number of fEPSPs persisting throughout anoxia. † Time to fEPSP abolition after introduction of anoxia. ‡ Number of fEPSPs reappearing during anoxia.

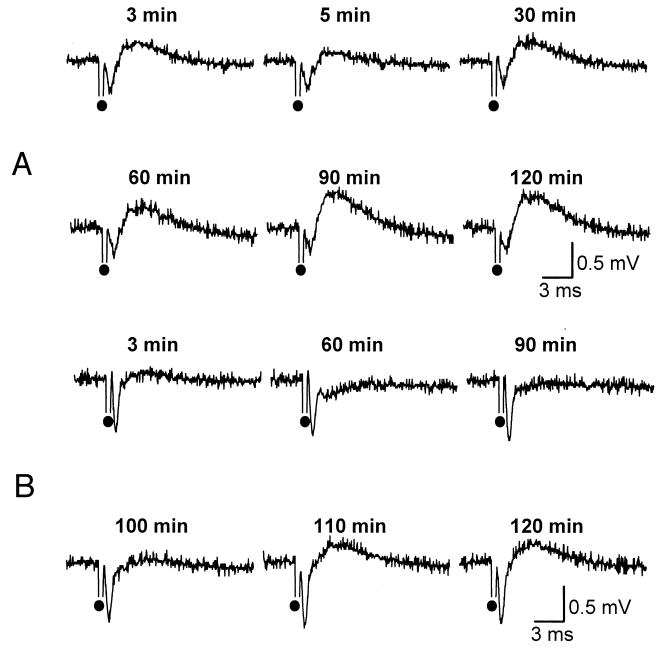

FIG. 5. Synaptic transmission partially supported by glycolysis during 120 min anoxia at 20 mM glucose in ACSF. *A*: persistence of PV and field excitatory postsynaptic potential (fEPSP). *B*: persistence of PV with loss and eventual reappearance of fEPSP. Numbers indicate time (min) after start of anoxia. ●, stimulation artifacts. Persistence of PV and fEPSP or the fEPSP reappearance during anoxia indicates that the synaptic transmission is partially maintained, and the glycolysis of glucose partially supported the synaptic transmission during such prolonged up to 120 min anoxia at 20 mM glucose in ACSF.

Recovery of synaptic transmission following anoxia

Table 2 summarizes the PS recovery after different durations of anoxia in the presence of different concentrations of glucose and combinations in ACSF.

Effect of glucose concentration on recovery of synaptic transmission following anoxia

When the slice was superfused with ACSF containing 4 mM glucose, following 10 min anoxia, although the PV recovered (Fig. 2*A*), PS displayed no recovery after 60 min reoxygenation (Fig. 2, *A* and *B*, and Table 2). This indicated that synaptic transmission was completely damaged by 10 min anoxia at 4 mM glucose in ACSF. However, when anoxia was terminated at 30 s after anoxic depolarization occurred, the PS slowly recovered within 2–7 min (4.2 ± 1.8 min, $n = 7$) after reoxygenation (Fig. 6, *A* and *B*). Its recovery at the end of 60 min reoxygenation was 34 ± 15% ($n = 7$). At 4 mM glucose in ACSF, the PS did partially recover when anoxia was terminated at 30 s after the occurrence of anoxic depolarization, indicating anoxic duration after the occurrence of anoxic de-

TABLE 2. *Effect of glucose concentration on PS recovery after anoxia*

Glucose, mM	Addition	Anoxia, min	*n*	Recovery Start, min*	Final Recovery, %†
4		10	6		0
10		10	6	3.0 ± 0.7	105 ± 14
10		60	7	3.0 ± 1.6	91 ± 27
20		60	6	2.1 ± 0.9	119 ± 25
20		120	6	1.8 ± 0.6	112 ± 12
4	16 mM mannitol	10	6		0
4	16 mM Na L-lactate	10	6		0
20	1 mM NaCN	120	6	2.5 ± 0.6	118 ± 9
10	0.5 mM 4-CIN	10	6	2.4 ± 0.3	107 ± 6
10	0.5 mM 4-CIN	60	6	2.3 ± 0.8	100 ± 13

Values in Recovery Start and Final Recovery are means ± SD; *n* is number of slices. PS, population spike; NaCN, sodium cyanide; Na L-lactate, sodium L-lactate; 4-CIN, α-cyano-4-hydroxycinnate. * Time of PS recovery after start of reoxygenation. † PS amplitude as percent of control at the end of 60 min reoxygenation.

polarization was strongly correlated with the degree of recovery of synaptic transmission following anoxic challenge.

When the slice was superfused with ACSF containing 10 mM glucose, following 10 min anoxia, the PS rapidly recovered within 2.0–4.0 min after reoxygenation (Fig. 3*B* and Table 2), and its recovery at the end of 60 min reoxygenation was 105 ± 14% (Fig. 3, *A* and *B*, and Table 2). This suggests that glucose at 10 mM in superfusion ACSF prevents damage to synaptic transmission by 10 min anoxia. Even following 60 min anoxia, the PS still recovered within 1.5–6.0 min after reoxygenation (Fig. 3*D* and Table 2), and its recovery at the end of 60 min reoxygenation was 91 ± 27% (Fig. 3, *C* and *D*, and Table 2). Thus even following 60 min anoxia, glucose at 10 mM in ACSF still protects synaptic transmission from irreversible damage.

When the slice was superfused with ACSF containing 20 mM glucose, following 60 min anoxia, the PS rapidly recovered within 1.0–3.5 min after reoxygenation (Fig. 4*B* and Table 2), and its recovery at the end of 60 min reoxygenation was 119 ± 25% (Fig. 4, *A* and *B*, and Table 2). Even following 120 min anoxia, the PS rapidly recovered within 1.0–2.5 min after reoxygenation as well (Fig. 4*D* and Table 2), and its recovery at the end of 60 min reoxygenation was 112 ± 12% (Fig. 4, *C* and *D*, and Table 2). This indicated that the 20 mM glucose provided virtually complete protection against anoxia-induced damage to synaptic transmission by prolonged anoxia even up to 120 min.

Effect of osmolality on recovery of synaptic transmission following anoxia

The protection of synaptic transmission during anoxia by higher glucose concentration could be due to higher osmolality in ACSF (Huang et al. 1996) rather than the glucose itself. To evaluate this possibility, 16 mM mannitol was added into ACSF containing 4 mM glucose to yield an ACSF with an osmolality equal to that of ACSF containing 20 mM glucose. When slices were superfused with this ACSF, following 10 min anoxia the PS still did not recover at all after 60 min reoxygenation (Fig. 6, *C* and *D*, and Table 2). These results indicated that the protection against anoxia-induced disruption of synaptic transmission was due to glucose itself, not to higher osmolality.

Effect of superfusion rate on recovery of synaptic transmission following anoxia

The superfusion rate varied from 1–2 ml/min (Khazipov et al. 1993; Shurr et al. 1987), 4–5 ml/min (Takata and Okada 1995), 7–9 ml/min (Fujiwara et al. 1987), 25–35 ml/min (Kass and Lipton 1982; Lipton and Whittingham 1982), up to 60–70 ml/min (Fried et al. 1995; Kass and Lipton 1986) at different laboratories. Because the anoxic depolarization is very transient in the slices (Fig. 2*C*), the potassium might be washed out at the superfusion rate of 7–8 ml/min. To evaluate this possibility, the slices were superfused at a slower flow rate. At ~2 ml/min superfusion rate, the results were similar to those at the superfusion rate of 7–8 ml/min. When slices were superfused with ACSF containing 20 mM glucose at ~2 ml/min (1.8–2.2 ml/min), following 120 min anoxia, the PS still rapidly recovered within 1.0–3.0 min after reoxygenation (Fig. 7*B*), and its recovery at the end of 60 min reoxygenation was 114 ± 19% ($n = 6$; Fig. 7, *A* and *B*). However, the PS was abolished at a slower rate after introduction of anoxia when slices were superfused at the slower flow rate (3.0–5.0 min vs. 2.0–3.0 min). This indicated that when the slice was superfused with ACSF containing 20 mM glucose, the superfusion rate did not affect recovery of synaptic transmission following 120 min anoxia.

Effect of metabolism on recovery of synaptic transmission following anoxia

Our recordings were made at least 150 μm below the upper surface of the slice. In this region during anoxia, no oxygen is available to neurons (Fujii et al. 1982; Fujiwara et al. 1987). To confirm the absence of oxygen during anoxic challenge, the following experiments were performed. First, if during anoxia the tissue was able to acquire some oxygen, lactate could be used to produce ATP via aerobic metabolism, and then provide some protective effect on synaptic transmission against anoxic damage. Therefore 16 mM sodium L-lactate was added into ACSF containing 4 mM glucose, to confirm whether aerobic metabolism of glucose during anoxia was responsible for the protection against anoxia-induced damage. With this ACSF, following 10 min anoxia, the PS failed to recover after 60 min reoxygenation (Fig. 7, *C* and *D*, and Table 2), suggesting that the production of ATP via aerobic metabolism of glucose during anoxia (with consequent protection against anoxia) was

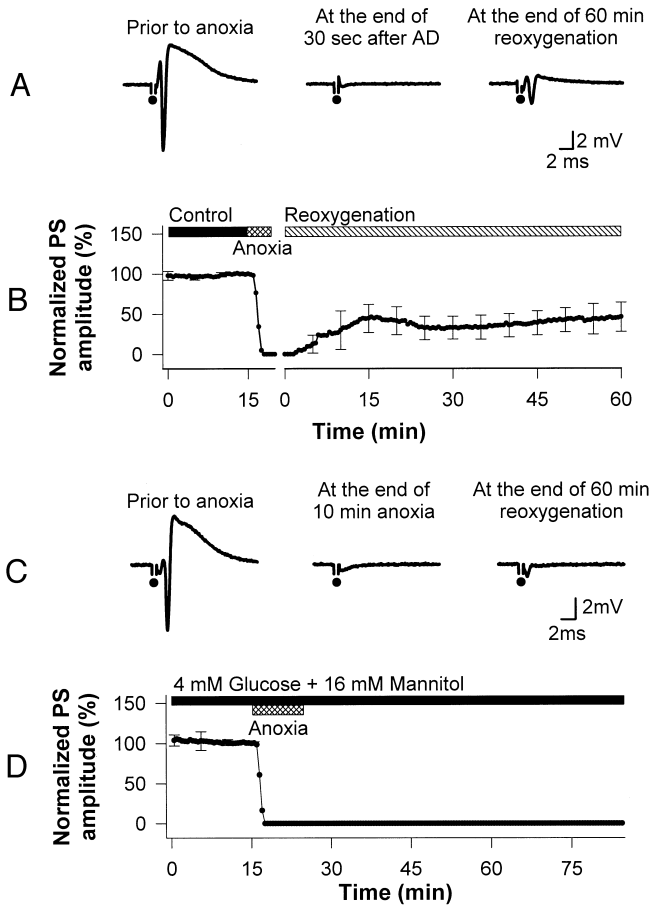

FIG. 6. Effects of anoxic duration and osmolality on recovery of synaptic transmission after anoxia terminated at 30 s after anoxic depolarization at 4 mM glucose (*A* and *B*) or 10 min anoxia at 4 mM glucose plus 16 mM mannitol (*C* and *D*) in ACSF. Recordings were obtained before anoxia, at the end of 30 s after anoxic depolarization (*A*) or 10 min anoxia (*C*), and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A* and *C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during anoxia terminated at 30 s after anoxic depolarization (*B*) or 10 min anoxia (*D*), and during 60 min reoxygenation. At 4 mM glucose in ACSF, the PS did partially recover when anoxia was terminated at 30 s after the occurrence of anoxic depolarization, indicating anoxic duration after the occurrence of anoxic depolarization was strongly correlated with the recovery of synaptic transmission after anoxic challenge. The PS did not recover in *C* and *D*, indicating irreversible damage to synaptic transmission by 10 min anoxia at 4 mM glucose plus 16 mM mannitol in ACSF and higher osmolality in higher glucose concentration in ACSF did not play an important role in glucose protection of synaptic transmission against anoxic damage. AD, anoxic depolarization. Error bars represent SD (5-min intervals).

not responsible for the protection against anoxia-induced damage. Second, 1 mM NaCN was added to ACSF containing 20 mM glucose during anoxia to cause anoxic anoxia combined with chemical anoxia (Wind et al. 1997). Before and after this combined anoxia, the slice was superfused with ACSF containing 20 mM glucose without NaCN. The results were similar to those during anoxia when slices were superfused with ACSF containing 20 mM glucose without 1.0 mM NaCN. The fEPSP either persisted or recovered during anoxia, and the PV persisted throughout the anoxic period of 120 min. After reoxygenation, the PS returned within 1.5–3.5 min, and its recovery at the end of 60 min reoxygenation was 118 ± 9% (Fig. 8, *A* and *B*, and Table 2). To confirm whether the cyanide in the bicarbonate/CO_2 ACSF endangered the slice preparation, the slice was challenged with only 1.0 mM NaCN chemical anoxia alone without anoxic anoxia for 10 min at 4 mM glucose. When the slice was superfused with ACSF containing 4 mM glucose, following 10 min 1.0 mM NaCN chemical anoxic challenge, the PS failed to recover after 60 min reoxygenation ($n = 6$; Fig. 8, *C* and *D*). Therefore the cyanide endangered the slice preparation, and 10 min 1.0 mM NaCN chemical anoxia alone without anoxic anoxia could completely damage the synaptic transmission in 4 mM glucose bicarbonate/CO_2 ACSF. These results also confirmed that the protection afforded by glucose was not due to ATP produced via aerobic metabolism during anoxic challenge.

The lactate produced by glucose during anoxia may play a very important role in the recovery of synaptic transmission after reoxygenation (Schurr et al. 1997a,b). When the slice was superfused with 10 mM glucose ACSF containing 0.5 mM 4-CIN (a lactate transporter inhibitor that inhibits the lactate

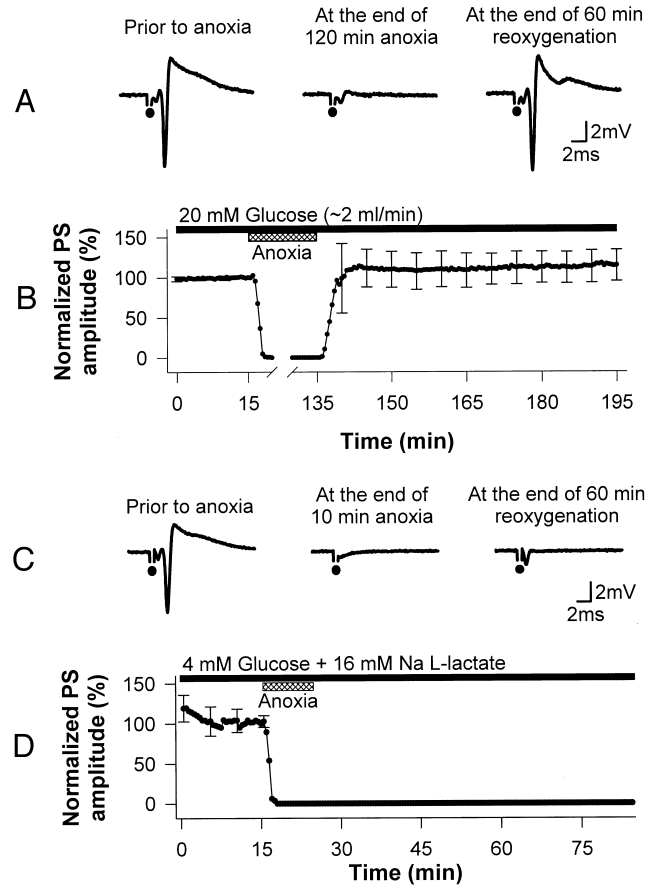

FIG. 7. Effects of superfusion rate and aerobic metabolism on recovery of synaptic transmission after 120 min anoxia at 20 mM glucose in ACSF (*A* and *B*) and 10 min anoxia at 4 mM glucose plus 16 mM sodium L-lactate (*C* and *D*) in ACSF. Recordings were obtained before anoxia, at the end of 120 min anoxia (*A*) or 10 min anoxia (*C*), and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A* and *C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during 120 min anoxia (*B*) or 10 min anoxia (*D*), and during 60 min reoxygenation in *B* and *D*. The PS was fully restored after 120 min anoxia at 20 mM glucose when the superfusion rate was ~2 ml/min (*A* and *B*). Results were similar to those at the superfusion rate of 7–8 ml/min, indicating that the superfusion rate does not affect recovery of synaptic transmission after anoxia. PS did not recover after 10 min anoxia at 4 mM glucose plus 16 mM sodium L-lactate in ACSF (*C* and *D*), indicating that there was no effect of aerobic metabolism on recovery of synaptic transmission after anoxia. Error bars represent SD (5-min intervals).

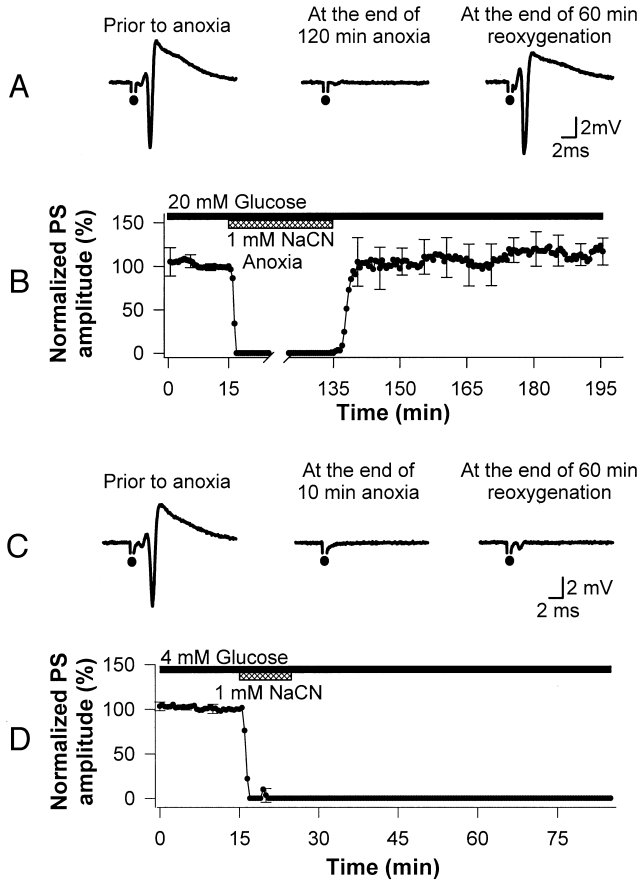

FIG. 8. Effects of chemic anoxia on recovery of synaptic transmission after 120 min combined anoxia (chemic anoxia combined with anoxic anoxia) at 20 mM glucose [during anoxia with addition of 1.0 mM NaCN (aerobic metabolic blocker); *A* and *B*] or 10 min chemic anoxia alone (1.0 mM NaCN without anoxic anoxia) at 4 mM glucose (*C* and *D*) in ACSF. Recordings were obtained before anoxia, at the end of 120 min combined anoxia (*A*) or 10 min chemic anoxia alone (*C*) and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A* and *C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during 120 min combined anoxia (*B*) or 10 min chemic anoxia alone (*D*), and during 60 min reoxygenation in *B* and *D*. PS was fully restored after 120 min anoxia at 20 mM glucose even during anoxia with addition of 1.0 mM NaCN in ACSF, indicating that there was no effect of aerobic metabolism on recovery of synaptic transmission after anoxia. PS did not recover after 10 min chemic anoxia alone at 4 mM glucose, indicating that 10 min 1.0 mM NaCN chemic anoxia alone could completely damage the synaptic transmission at 4 mM glucose in ACSF. Error bars represent SD (5-min intervals).

transportation from glia to neurons), the results were similar to those when slices were superfused with ACSF containing 10 mM glucose without 0.5 mM 4-CIN. The PV persisted throughout the anoxic period, and the fEPSP either persisted or reappeared during anoxia. The PS returned after reoxygenation, and their recoveries at the end of 60 min reoxygenation were 107 ± 6% and 100 ± 13% following 10 and 60 min anoxia, respectively (Fig. 9 and Table 2). These results indicate that the protection of glucose is not due to lactate produced by glia, but is due to glucose metabolism by the neuron itself.

DISCUSSION

During anoxia, neuronal energy supply decreases because neurons cannot produce energy via efficient oxidative metabolism; to compensate, there is a rapid increase in anaerobic metabolism (Duffy and Pulsinelli 1979; Lutz and Nilsson 1997). During anoxia, brain tissue requires more glucose via glycolysis to compensate for the inefficiency of anaerobic metabolism, because 1 molecule of glucose produces only 2 molecules of ATP via anaerobic metabolism, whereas 1 molecule of glucose produces 38 molecules of ATP via aerobic metabolism (Lutz and Nilsson 1997; Schurr et al. 1997a; Wass and Lanier 1996). Thus we hypothesized that glucose in elevated supply would provide a sufficient source of energy via glycolysis to preserve important neuronal function during anoxia. There is precedence for this phenomenon in turtle brain tissue (Lutz 1992; Lutz and Nilsson 1997).

General inhibition to synaptic transmission by anoxia

Our results showed that the PS amplitude began to decrease ~0.5–1.5 min after the introduction of anoxia. This decrease might be caused by a decrease in ATP in the synaptic region of the hippocampal slice, i.e., in the molecular layer (Lipton and

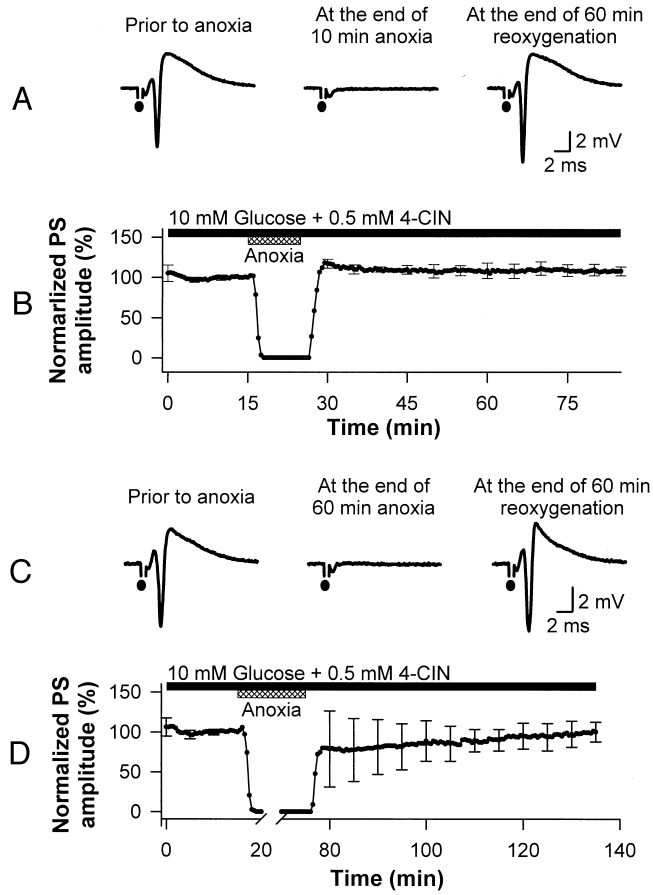

FIG. 9. Effect of lactate produced by glia on recovery of synaptic transmission after 10 min anoxia (*A* and *B*) or 60 min anoxia (*C* and *D*) at 10 mM glucose plus 0.5 mM α-cyano-4-hydroxycinnate (4-CIN) in ACSF. Recordings were obtained before anoxia, at the end of 10 min (*A*) or 60 min (*C*) anoxia, and at the end of 60 min reoxygenation in *A* and *C*. ● (in *A* and *C*), stimulation artifacts. Normalized PS amplitudes were obtained during 15 min before anoxia, during 10 min anoxia (*B*) or 60 min anoxia (*D*), and during 60 min reoxygenation in *B* and *D*. After 10 min anoxia or 60 min anoxia, the PS recoveries were similar to those in 10 mM glucose ACSF without 0.5 4-CIN (Fig. 2), indicating that lactate produced by glia might not play an important role in the glucose protection of synaptic transmission against anoxic damage in this study. Error bars represent SD (5-min intervals).

Whittingham 1982), and the increase in extracellular K^+ due to inhibition of the Na^+/K^+ ATP pump (Fujiwara et al. 1987; Lipton and Whittingham 1979). The PS was completely abolished ~1.5–3.0 min after the exposure to anoxia. This effect might be due to a neuronal depolarization because extracellular K^+ increased (Fujiwara et al. 1987; Lipton and Whittingham 1979).

Damage to synaptic transmission by anoxia

When the hippocampal slice was superfused with ACSF containing 4 mM glucose, there was no recovery of the PS following exposure to anoxia for only 10 min. This finding indicates that synaptic transmission in CA1 is completely damaged by 10 min exposure of anoxia under these conditions, a result consistent with those of Kass and Lipton (1986, 1989). This irreversible damage to synaptic transmission might be due to both a decreased ATP level and an increased cytosolic calcium level (Kass and Lipton 1982, 1986, 1989). However, at 4 mM glucose in ACSF, when anoxia was terminated at 30 s after anoxic depolarization occurred, the PS could partially recover after reoxygenation. When the slice was superfused with 4 mM glucose ACSF, the anoxic depolarization always occurred after anoxia. The anoxic duration after anoxic depolarization was 5–8 min (Fig. 1*C*) when the slice was challenged by 10 min anoxia at 4 mM glucose in ACSF, so the ion gradients across the neuronal membrane might be severely disturbed. Our results further confirm that the anoxic duration after anoxic depolarization is strongly correlated with the irreversible transmission damage (Roberts and Sick 1988; Sick et al. 1987).

Glucose protection against damage to synaptic transmission by anoxia

Although previous reports have stated that elevated glucose improves functional recovery from anoxic challenge in vitro (Grigg and Anderson 1989; Schurr et al. 1987), it was unpredicted and remarkable in the present study to find that glucose offers such an extremely powerful protection against damage to synaptic transmission by anoxia in vitro.

During 60 min and even 120 min of anoxia, when the slice was superfused with ACSF containing 20 mM glucose, the evoked PV and fEPSP could be sustained throughout the duration of anoxia (Fig. 4*A*). This finding indicated that during up to 120 min of exposure to anoxia, the presynaptic terminal could still be excited and could retain the capability to release neurotransmitters, and the postsynaptic receptors could still be excited by those neurotransmitters. These novel findings in the anoxic hippocampal slice demonstrated that mammalian neuronal synaptic functions could be sustained under severe anoxic conditions if there were enough glucose in the brain tissue. As pointed out previously, these conditions are somewhat like those of turtle brain tissue (Lutz 1992; Lutz and Nilsson 1997), which can survive a prolonged anoxic challenge. During anoxia there is a marked hyperglycemia in the turtle, with plasma glucose rising to as much as 17.7 mM (Penney 1974) accompanied by an increase in blood flow to the brain by as much as 260% (Davies 1990). During prolonged anoxia (up to 6 h), extracellular potassium concentration $[K^+]_o$ remained near baseline in intact turtle brain (Sick et al. 1982). In isolated turtle cerebellum, with 20 mM glucose in ACSF, ATP levels could be maintained and significant increase in $[K^+]_o$ could be prevented during prolonged anoxia (up to 6 h) (Perez-Pinzon et al. 1992). Although evoked field potentials were significantly depressed at the end of 4 h anoxia, evoked field potentials could restore to the initial values (Perez-Pinzon et al. 1992).

However, in most of our hippocampal slices, during anoxia the fEPSP was restored after its disappearance at the beginning of the anoxia (Fig. 5*B*). We interpreted this observation as follows. At the beginning of anoxia, the brain tissue cannot produce enough high-energy phosphates such as ATP to sustain the fEPSP. The glucose anaerobic metabolism pathway, which might be inhibited in normoxia, becomes more active as anoxia progresses. It has been suggested that this Pasteur effect, i.e., increased glycolysis as compensation for inhibition of oxidative metabolism, may exist during anoxia in turtle brains (Lutz 1992; Perez-Pinzon et al. 1992). Therefore the fEPSP progressively recovered after its initial disappearance (Fig. 5*B*). When the brain tissue produced enough high-energy phosphates to support synaptic functions, the fEPSP was slowly increased or restored (Fig. 5, *A* and *B*).

Our results demonstrated that higher concentrations of glucose prevented damage to synaptic transmission by anoxia, resulting in faster and complete recovery of PS amplitudes when the slices were reoxygenated. Indeed, 20 mM glucose provides virtually complete protection against damage to synaptic transmission by anoxia lasting as long as 120 min. This in vitro powerful protection against anoxia-induced synaptic transmission damage is impressive. Synaptic transmission in our slices was impaired less compared with the results of an earlier report (Schurr et al. 1987). In that study, only 39 and 93% of hippocampal slices recovered synaptic function following 10 min anoxia in the presence of 10 and 20 mM glucose, respectively. In our study, following 10 min anoxia, 100% of slices recovered synaptic function and the PS amplitude recovered to a mean of 105% of preanoxic value at 10 mM glucose. The effect of the superfusion rate on these results was evaluated. The results at the superfusion rate of ~2 ml/min were similar to those at the superfusion rate of 7–8 ml/min (Fig. 7, *A* and *B*), indicating that the superfusion rate does not affect recovery of synaptic transmission following anoxia. The better "performance" of our tissues might be due to the following factors. First, the hippocampal slices in this study may have less traumatic injury (Lipton et al. 1995), because our slices were obtained by a vibratome slicer and cutting speed was very slow (<5 mm/min), whereas theirs were obtained by McIIwain chopper. Second, the hippocampal slices were obtained from younger rats in this study (weighing 150–200 g vs. 200–350 g). Finally, there were differences in the method of interpreting recovery of synaptic function. This study compared the PS amplitude at the end of 60 min reoxygenation of each slice to its own preanoxic level.

Mechanism of glucose protection against anoxic synaptic transmission damage

PROTECTIVE EFFECT OF HIGH OSMOLALITY ON ANOXIA SYNAPTIC TRANSMISSION DAMAGE. Although it has been shown that when 100 mM mannitol or fructose was added into 10 mM glucose ACSF, such a hyperosmolality environment

improved functional recovery from anoxia (Huang et al. 1996), in our study, 4 mM glucose plus 16 mM mannitol or 16 mM sodium L-lactate in ACSF did not show any protection against 10 min anoxic damage to synaptic transmission. In contrast, 10 mM glucose and 20 mM glucose in ACSF provided complete protection against 10 and 120 min anoxic damage to synaptic transmission, respectively. These results therefore confirmed that the protection offered by high concentrations of glucose against damage to synaptic transmission by anoxia was not due to an increase in osmolality.

OTHER SOURCES OF OXYGEN IN THE PREPARATION. Our recordings were performed at depths of at least 150 μm from the upper surface of the hippocampal slice so that in the recording area there was no oxygen that could be used by neurons (Fujii et al. 1982; Fujiwara et al. 1987). Furthermore, if neurons could use the extra glucose to produce energy via oxidative metabolism, then they could certainly use lactate to produce energy via oxidative metabolism to protect synaptic transmission from anoxic damage. However, there was no recovery of the PS when the hippocampal slice was perfused with an ACSF containing 4 mM glucose and 16 mM Na^+ L-lactate.

It should also be noted that, if oxygen from other sources was used, oxidative metabolism should be blocked during chemical anoxia combined with anoxic anoxia; i.e., when the ACSF contained 1 mM NaCN (Wind et al. 1997). At 4 mM glucose in ACSF, without anoxic anoxia, 10 min NaCN chemical anoxia alone could completely damage the synaptic transmission (Fig. 8, *C* and *D*). However, there was no synaptic transmission damage even following a 120-min exposure to chemical anoxia combined with anoxic anoxia in the presence of 20 mM glucose in ACSF (Fig. 8, *A* and *B*).

These results therefore demonstrate that the protection high concentration glucose affords against damage to synaptic transmission by anoxia is not due to any glucose oxidative metabolism during anoxia.

ROLE OF LACTATE PRODUCED BY GLIA IN SYNAPTIC TRANSMISSION RECOVERY. The lactate produced by glia might be an obligatory aerobic energy substrate for functional recovery after anoxia (Schurr et al. 1997a,b). In this study, when 0.5 mM 4-CIN was added into 10 mM glucose ACSF, the lactate transportation was inhibited by 4-CIN (Izumi et al. 1997). However, the recoveries of synaptic transmission following 10 and 60 min anoxia were similar to those at 10 mM glucose without 4-CIN in ACSF. Therefore the lactate produced by glia might not play an important role in the synaptic transmission recovery following anoxia in this study.

These findings confirmed that the protection offered by high concentrations of glucose against damage to synaptic transmission during anoxia in vitro is due to the anaerobic metabolism of glucose.

In summary, three novel findings have been reported in this study. First, the evoked PV, representing action potentials of presynaptic axonal terminal, could be elicited throughout 120 min anoxia in the presence of 20 mM glucose with or without 1.0 mM NaCN in ACSF. Thus with 20 mM glucose in ACSF, the excitability of the presynaptic axonal terminal is not impaired by prolonged anoxia (up to 120 min). Second, during superfusion with ACSF containing 20 mM glucose with or without 1.0 mM NaCN, the evoked fEPSP either persisted or recovered during prolonged anoxia (up to 120 min). Therefore with 20 mM glucose in ACSF, synaptic transmission could be partially sustained by glycolysis during anoxia lasting as long as 120 min. Third, we demonstrated that the lack of glucose is the major cause of anoxia-induced damage to synaptic transmission, and glucose, via in vitro anaerobic metabolism, provides powerful protection against prolonged up to 120 min anoxia-induced damage of synaptic transmission.

We conclude that lack of glucose is the major cause of anoxia-induced synaptic transmission damage, and that if sufficient glucose is supplied, this damage could be prevented by glycolysis in vitro.

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