2-Deoxyglucose–Induced Long-Term Potentiation in CA1 Is Not Prevented by Intraneuronal Chelator

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Zhao, Yong-Tao and Krešimir Krnjević. 2-Deoxyglucose–induced long-term potentiation in CA1 is not prevented by intraneuronal chelator. J. Neurophysiol. 83: 177–180, 2000. In hippocampal slices, temporary (10–20 min) replacement of glucose with 10 mM 2-deoxyglucose is followed by marked and very sustained potentiation of EPSPs (2-DG LTP). To investigate its mechanism, we examined 2-DG’s effect in CA1 neurons recorded with sharp 3 M KCl electrodes containing a strong chelator, 50 or 100 mM ethylene glycol-β-aminoethyl ether)-N,N,N’,N’'-tetraacetic acid (EGTA). In most cases, field EPSPs were simultaneously recorded and conventional LTP was also elicited in some cells by tetanic stimulation of stratum radiatum. 2-DG potentiated intracellular EPSP slopes by 48 ± 5.1% (SE) in nine cells recorded with plain KCl electrodes and by 52 ± 6.2% in seven cells recorded with EGTA-containing electrodes. In four of the latter cells, tetanic stimulation (twice 10 Hz for 1 s) failed to evoke LTP (2 ± 1.1%), although field EPSPs were clearly potentiated (by 28 ± 6.9%). Thus unlike tetanic LTP, 2-DG LTP is not readily prevented by postsynaptic intraneuronal injection of EGTA. These findings agree with other evidence that the rise in postsynaptic (somatic) [Ca²⁺]i caused by 2-DG is not the principal trigger for the subsequent 2-DG LTP and that it may be a purely presynaptic phenomenon.

INTRODUCTION

Although there is much disagreement about underlying mechanisms, it is widely agreed that the induction of conventional long-term potentiation (LTP) is initiated by a rise in postsynaptic intracellular Ca²⁺ concentration ([Ca²⁺]i) (Baudry 1998; Bliss and Collingridge 1993; Larkman and Jack 1995; Nicoll and Malenka 1995). The principal evidence for this is the finding that intraneuronal injections of a strong chelator, such as ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), consistently prevent the subsequent induction of LTP induced by tetanic stimulation in CA1 pyramidal cells (Lynch et al. 1983; Parfitt and Madison 1993) and other types of neurons (Komatsu and Iwakiri 1992; Ouardouz and Lacaille 1995; Shindou et al. 1993). Even some unconventional forms of LTP can be prevented by similar postsynaptic injections of chelators. For example, LTP induced by NMDA receptor-independent Ca²⁺ influx (Kullmann et al. 1992) and selective LTP of the N-methyl-d-aspartate (NMDA) receptor component of EPSPs induced by anoxia combined with aglycemia (Crépel and Ben-Ari 1996). By contrast, the LTP of mossy fiber EPSPs, which is believed to have a presynaptic mechanism, is not sensitive to postsynaptic injections of chelators (Katsuki et al. 1991; Zalutsky and Nicoll 1990).

Another unconventional type of LTP is one produced very reliably in CA1 neurons by 2-deoxyglucose (Tekkók and Krnjević 1995), which blocks glycolysis by competing with glucose for phosphorylation by hexokinase (Tower 1958). Albeit NMDA receptor-dependent, this form of LTP (2-DG LTP) is very atypical in that 2-DG causes postsynaptic hyperpolarization, not depolarization (Zhao et al. 1997). Nevertheless, it is also Ca²⁺-dependent (Tekkók and Krnjević 1996) and 2-DG raises [Ca²⁺], in hippocampal neurons (Tekkók et al. 1999). It was therefore of interest to see whether similar injections of EGTA into CA1 pyramidal cells would also prevent the subsequent induction of 2-DG LTP. A preliminary report of these results has appeared as an abstract (Zhao and Krnjević 1997).

METHODS

Young Sprague–Dawley male rats (110–180 g) obtained from Charles River, Québec, were decapitated under full halothane anesthesia. The brain was quickly removed and the hippocampus dissected out in ice-cold oxygenated saline. Transverse slices (400 μm thick) were cut with a Vibroslice (Campden Instruments, Loughborough, U. K.). They were kept for 1 h at room temperature in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3.0 KCl, 2.0 CaCl₂, 2.0 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose (pH ~7.3) and were continually aerated with carbogen (95% O₂–5% CO₂). Slices were then transferred to a recording chamber where they were submerged under 0.1–0.2 mm of flowing carbogenated ACSF and kept at 34 ± 0.5°C (SE).

The sharp microelectrodes were pulled from thin-walled borosilicate glass tubes (1.2 mm OD, WP Instruments, New Haven, CT). After being filled with 3 M KCl, the electrodes had resistances of 60–80 MΩ. To inject the chelator by diffusion into neurons, 50 or 100 mM EGTA (Sigma, St. Louis, MO) was added to the 3 M KCl electrode-motor solution. To prevent distortion of EPSPs by spiking, 10 mM QX-222 or QX-314 (Astra Pharma, Ontario) was also added to many of the electrodes.

Field recordings from CA1 stratum radiatum were made with low resistance 2 M NaCl-containing electrodes. Half-maximal synaptic responses were evoked by stimuli applied at intervals of 20 s through insulated nickel-chromium wires placed in the s. radiatum. The signals were amplified by an Axoclamp 2 (Axon Instruments, Burlington, CA) in current-clamp mode.

Tetanic LTP was elicited by s. radiatum stimulation with two 100 Hz volleys, each lasting 1 s and repeated after 20 s. 2-Deoxy-D-glucose (2-DG, Sigma) was applied for 10–20 min by equimolar replacement of 10 mM glucose in ASCF.

Means ± SE are given throughout. The significance of differences between means was assessed by Student’s t-test (whenever possible for paired results).

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RESULTS

Intracellular penetrations into the stratum pyramidale of CA1 yielded stable recordings from nine control neurons with plain 3 M KCl electrodes and from seven test neurons with similar KCl electrodes containing 50 or 100 mM EGTA.

Effects of 2-DG

ACSF glucose was replaced by 2-DG for periods of 10–20 min, which regularly elicit 2-DG LTP in field recordings. The mean times were not significantly different \( (P = 0.11) \) for control cells \((15 \pm 1.2 \text{ min})\) and EGTA-injected cells \((13 \pm 0.8 \text{ min})\).

ON MEMBRANE POTENTIAL. Initially, the resting potentials were similar in the two groups: \(-68.6 \pm 0.41 \text{ mV}\) for the nine control cells and \(-68.0 \pm 0.58 \text{ mV}\) for the seven EGTA-injected cells. However, in contrast to the control cells, which were significantly hyperpolarized during the 2-DG applications \((4.9 \pm 1.69 \text{ mV}; \ P = 0.019)\), the EGTA-injected cells showed no significant change in potential \((-1.0 \pm 0.58 \text{ mV})\).

ON EPSPS. As observed earlier (Tekkök and Krnjević 1995, 1996; Zhao et al. 1997), 2-DG had a biphasic effect; after a 10 min delay EPSPs were sharply reduced, reaching a minimum shortly after the return of glucose. This was followed by recovery and then a well-sustained potentiation (lasting >30 min). In nine control cells recorded with plain KCl electrodes, EPSPs were enhanced by 48 ± 5.14%. As illustrated in Fig. 1, simultaneously recorded field EPSPs showed quite comparable changes. In three such double recordings, the LTPs of field and intracellular EPSPs differed by only 2.7 ± 2.9% (paired data).

In seven cells recorded with electrodes containing 50 or 100 mM EGTA (as well as 3 M KCl) the 2-DG applications resulted in sustained potentiation by 52 ± 6.2%. The LTP in simultaneous field recordings was virtually identical \((-1.1 \pm 4.0\%, \ n = 7; \ \text{paired data})\) as can be seen in Figs. 2 and 3. As illustrated in these figures, tetanic stimulation failed to induce any LTP of intracellular EPSPs \((2 \pm 1.1\%, \ n = 4)\) though in the same slices the corresponding field EPSPs were potentiated by 28 ± 6.9% \((P < 0.05)\). However, though EGTA did not prevent 2-DG LTP of intracellular EPSPs, subsequent low-frequency stimulation did not elicit depotentiation (Fig. 3).

DISCUSSION

The main findings can be summarized as follows. The extracellular recordings showed quite conventional LTP after both tetanic stimulation and applications of 2-DG, which could be depotentiated by low-frequency stimulation. In intracellular recordings with plain KCl-containing sharp electrodes, similar changes were seen as well as the characteristic membrane hyperpolarization elicited by 2-DG (Zhao et al. 1997). However, in cells recorded with electrodes also containing EGTA, though sufficient EGTA leaked to prevent 1) significant tetanic LTP, 2) 2-DG-induced hyperpolarization, and 3) depotentiation of 2-DG LTP by low-frequency stimulation, nevertheless marked LTP was consistently induced by 2-DG.

These results are fully in agreement with previous studies that diffusion of EGTA from recording microelectrodes readily prevents the tetanic induction of either LTP (Lynch et al. 1983; Parfitt and Madison 1993) or LTD (Domenici et al. 1998; Oliet et al. 1997); from which it has been widely accepted that the sustained changes in CA1 EPSPs induced tetanically are triggered by a rise in postsynaptic \([\text{Ca}^{2+}]_{\text{i}}\) (Baudry 1998; Bliss and Collingridge 1993; Larkman and Jack 1995; Nicoll and Malenka 1995). Although the precise location of the critical rise in \([\text{Ca}^{2+}]_{\text{i}}\) is not known, it must be readily accessible to EGTA leaking from a somatically-located sharp microelectrode. This is evidently not the case where 2-DG LTP is concerned.
These results are thus in keeping with various tests on the 2-DG-evoked rise in somatic \([\text{Ca}^{2+}]_{i}\), which revealed some major discrepancies between its properties and those of 2-DG LTP (Tekkök et al. 1999), and therefore led to the conclusion that the observed \([\text{Ca}^{2+}]_{i}\) rise cannot be essential for the LTP. And yet 2-DG LTP does appear to be \([\text{Ca}^{2+}]_{i}\)-dependent, being prevented by very prolonged lack of \([\text{Ca}^{2+}]_{i}\) or by dantrolene (Tekkök and Krnjević 1996). If the significant \([\text{Ca}^{2+}]_{i}\) change is not readily accessed by somatic injections of EGTA, where can it be situated?

It might be argued that it occurs in small dendritic branches/spines, too far to be easily reached by diffusion of EGTA from the soma; but this must be the site of most of the NMDA receptor-mediated \([\text{Ca}^{2+}]_{i}\) influx responsible for tetanic LTP, which is prevented by EGTA. A more likely explanation is that the critical events occur elsewhere, such as in adjacent glia or presynaptic terminals.

There is evidence that glia may alter the function of neighboring neurons by a \([\text{Ca}^{2+}]_{i}\)-dependent release of arachidonic acid and subsequent potentiation of glutamatergic transmission.

**FIG. 2.** When intracellular electrode contains EGTA, tetani evoke LTP only of field EPSP, but 2-DG LTP is seen in both extra- and intracellular recordings. Tetanic stimulation (100 Hz for 1 s, repeated once after 20 s) elicited usual LTP of field EPSPs (○) but no LTP of intracellular EPSP (●). (Note similar LTP of both field and intracellular EPSPs after 12 min of 2-DG.) \(V_m\) was kept at \(-70\) mV throughout. *Inset traces* were obtained at times indicated. Intracellular electrode contained 3 M KCl + 100 mM EGTA.

**FIG. 3.** Intracellular EGTA does not prevent 2-DG LTP of intracellular EPSPs, but latter show no tetanic LTP and no depotentiation. With 100 mM EGTA and 3 M KCl in electrode, 2-DG (16 min) induced LTP of both field (○) and intracellular EPSPs (●). After reducing EPSPs (by lowering stimulus intensity, before trace e), tetani elicited LTP only of field EPSPs. Later, 1-Hz stimulation depotentiates field EPSPs but not 2-DG-potentiated intracellular EPSPs. \(V_m\) was kept at \(-68\) mV throughout.
(Glowinski et al. 1994). A comparable interaction, initiated by 2-DG-induced glutamate leakage and ATP depletion (Tower 1958) could conceivably induce 2-DG LTP.

In view of the absence of NMDA receptors on most glia (Steinhauser et al. 1994), a presynaptic event is more likely. Our previous finding that 2-DG LTP virtually abolishes paired-pulse facilitation (Tekkök and Krnjević 1997) suggested that 2-DG LTP is expressed presynaptically. A simple explanation is that LTP is both induced and expressed in afferent terminals, the induction being mediated by a Ca\(^{2+}\)-dependent dantrolene-sensitive mechanism, necessarily inaccessible to postsynaptically EGTA.

There is an obvious parallel with the tetanic LTP of mossy fiber EPSPs, which is also independent of postsynaptic depolarization and is not affected by injecting chelators into the postsynaptic neurons (Katsuki et al. 1991; Nicoll and Malenka 1995; Zalutsky and Nicoll 1990). This form of LTP thus appears to be an essentially presynaptic phenomenon, initiated by a rise in intraterminal [Ca\(^{2+}\)] and activation of a Ca\(^{2+}\)-sensitive protein kinase A (PKA) (Nicoll and Malenka 1995; Villacres et al. 1998; Xiang et al. 1994). The mechanism of 2-DG LTP, however, cannot be identical because 2-DG LTP does not seem to involve PKA (Tekkök and Krnjević 1997) and it is NMDA receptor-dependent.

A likely target for a presynaptic action of glutamate is syntaxin, a protein that combines a role in synaptic vesicle docking (Bennett et al. 1992) with NMDA receptor properties (Smirnova et al. 1993). One can speculate that 2-DG LTP is induced by the conjunction of several critical events initiated by 2-DG: glutamate release and presynaptic NMDA receptor-triggered Ca\(^{2+}\) influx; intraterminal Ca\(^{2+}\) release from a dantrolene-sensitive store (Katchman and Hershkowitz 1993); and rapid depletion of ATP, which together initiate lasting changes in phosphorylation at a site involved in transmitter release.

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REFERENCES


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