Induction of LTD in the Dentate Gyrus In Vitro Is NMDA Receptor Independent, but Dependent on Ca$^{2+}$ Influx via Low-Voltage–Activated Ca$^{2+}$ Channels and Release of Ca$^{2+}$ From Intracellular Stores

YUE WANG, MICHAEL J. ROWAN, AND ROGER ANWYL

Departments of Physiology and Pharmacology and Therapeutics, Trinity College, Dublin 2, Ireland

Wang, Yue, Michael J. Rowan, and Roger Anwyl. Induction of LTD in the dentate gyrus in vitro is NMDA receptor independent, but dependent on Ca$^{2+}$ influx via low-voltage-activated Ca$^{2+}$ channels and release of Ca$^{2+}$ from intracellular stores. J. Neurophysiol. 77: 812–825, 1997. The mechanisms of the induction of long-term depression (LTD) of field excitatory postsynaptic potentials (EPSPs) and whole cell patch-clamped excitatory postsynaptic currents (EPSCs) were studied in the dentate gyrus of the rat hippocampus. LTD of field EPSPs measuring 40% of control at 30 min poststimulation was induced by low-frequency stimulation consisting of 900 pulses at 1 Hz. LTD of EPSCs measuring 37% of control was induced by a pairing procedure consisting of 60 pulses at 1 Hz applied under voltage clamp at a holding potential of −40 mV. The induction of LTD of field EPSPs was dependent on an influx of extracellular calcium, being reduced in a low-Ca$^{2+}$ (0.8 mM) medium. However, substantial LTD (26%) was still induced in such a medium, demonstrating the relatively low sensitivity of LTD induction to the level of extracellular Ca$^{2+}$. A high concentration of the N-methyl-d-aspartate receptor antagonist d(-)-2-amino-5-phosphonopentanoic acid (d-AP5) (100 μM) did not significantly inhibit the induction of LTD of EPSCs evoked by the intracellular pairing procedure. d-AP5 partially reduced the magnitude of LTD of field EPSPs, but substantial LTD was still induced in the presence of AP5. The induction of LTD was strongly inhibited by Ni$^{2+}$ (50 μM) but not by nifedipine (10 μM), indicating that Ca$^{2+}$ influx via T-type, but not L-type, Ca$^{2+}$ channels is required for the induction of LTD. The induction of LTD was strongly inhibited by thapsigargin, an agent known to deplete intracellular Ca$^{2+}$ stores. The induction of LTD, but not long-term potentiation (LTP), was also strongly inhibited by ruthenium red, an agent known to block the ryanodine receptors located on intracellular Ca$^{2+}$ stores. These results demonstrate that Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores is required for the induction of LTD, but not LTP. The results of the present experiments suggest that the induction of LTD involves the entry of Ca$^{2+}$ via low-voltage-activated voltage-gated Ca$^{2+}$ channels followed by release of Ca$^{2+}$ from intracellular ryanodine-receptor-sensitive Ca$^{2+}$ stores.

INTRODUCTION

Long-term depression (LTD) is a long-lasting activity-dependent reduction in excitatory glutamatergic transmission that can be induced by a period of low-frequency stimulation (LFS) (Bear and Malenka 1994; Linden 1994). In hippocampal slices, homosynaptic LTD is induced by a brief period of LFS at 1–10 Hz (Dudek and Bear 1992; Fuji et al. 1991; Mulkey and Malenka 1992). An increase in the intracellular concentration of Ca$^{2+}$ has been shown to be necessary for the induction of LTD in the hippocampus: it has been shown that buffering intracellular Ca$^{2+}$ to very low levels with Ca$^{2+}$ chelators prevented the induction of LTD (Bolshakov and Siegelbaum 1994; Mulkey and Malenka 1992). An elevation of the intracellular Ca$^{2+}$ concentration can be produced by Ca$^{2+}$ influx across the plasma membrane. Several studies have speculated that calcium influx occurs via N-methyl-d-aspartate receptors (NMDARs), because the NMDAR antagonist (-)-2-amino-5-phosphonopentanoic acid (AP5) was found to block the induction of homosynaptic LTD and depotentiation in the CA1 region of the hippocampus (Debanne et al. 1994; Dudek and Bear 1992; Fuji et al. 1991; Mulkey and Malenka 1992; O’Dell and Kandel 1994; Selig et al. 1995; Thiels et al. 1994; Xiao et al. 1995). However, such an AP5 block of LTD/depotentiation has not been substantiated in other studies in either CA1 (Bashir and Collingridge 1994; Bolshakov and Siegelbaum 1994; Yang et al. 1994) or the dentate gyrus (O’Mara et al. 1995a,b). Evidence for Ca$^{2+}$ influx via L-type voltage-gated Ca$^{2+}$ channels being necessary for LTD induction in CA1 has also been presented by Bolshakov and Siegelbaum (1994), with the antagonist nifedipine inhibiting the LTD induction. However, involvement of L-type Ca$^{2+}$ channels in induction of LTD was not found in other studies, because the antagonists nifedipine (Mulkey and Malenka 1992; Selig et al. 1995) and nimodipine (Bashir and Collingridge 1994) did not block LTD/depotentiation induction.

An alternative way in which intracellular Ca$^{2+}$ can be elevated is by release from intracellular stores. In a previous paper we presented evidence for a role of intracellular Ca$^{2+}$ release in the induction of LTD; dantrolene, which prevents Ca$^{2+}$ release from intracellular stores, blocked the induction of LTD (O’Mara et al. 1995a).

In the present study we investigate the mechanisms underlying the induction of LTD in the dentate gyrus, with the emphasis on elucidating the mechanisms by which intracellular Ca$^{2+}$ is increased. The induction of LTD has been studied in the presence of selective blockers of Ca$^{2+}$ channels, such as Ni$^{2+}$, nifedipine, and d-AP5, and also in the presence of several agents that are known to affect the intracellular storage of Ca$^{2+}$. These include ruthenium red, which inhibits opening of the ryanodine receptor Ca$^{2+}$ channel (Heinzi and MacDermott 1992; Ma et al. 1988), 4-chloro-3-ethylphenol, a novel agonist at the ryanodine receptor that releases Ca$^{2+}$ from a thapsigargin-sensitive pool (Larini et al. 1995), and thapsigargin, which depletes Ca$^{2+}$ stores (Thastrup et al. 1990).
METHO DS

All experiments were carried out on transverse slices of the rat hippocampus (weight 80–120 g) as previously described (O’Connor et al. 1995). The brains were rapidly removed after decapitation and placed in cold oxygenated (95% O2–5% CO2) medium. Slices were cut at a thickness of 350 μm with the use of a Campden vibroslice, and placed in a holding chamber containing oxygenated medium at room temperature. The slices were then transferred as required to a recording chamber for submerged slices and continuously superfused at a rate of 8 ml/min at 30–32°C.

The control medium contained (in mM) 120 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2.0 MgSO4, 2.0 CaCl2, and 10 d-glucose. All solutions contained 100 μM picrotoxin (Sigma) to block γ-aminobutyric acid-A-mediated activity. Additional drugs used were ruthenium red (Calbiochem), thapsigargin (Sigma), d-AP5 (Tocris Cookson), and 4-chloro-3-ethylphenol (Aldrich).

The patch-clamp electrode (resistance 5–8 MΩ) contained (in mM) 130 potassium gluconate, 10 KCl, 10 ethylene glycol-bis(β-aminobutyric ether)-N,N,N′,N′-tetraacetic acid, 1 CaCl2, 3 MgCl2, 20 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 2 MgATP, 0.5 NaGTP, and 5 QX 314, pH adjusted to 7.2 with KOH. Whole cell recordings from dentate granule cells were made with the use of an Axopatch 1D amplifier (3-kHz low-pass Bessel filter). The capacitive current was always electronically cancelled and the series resistance (8–20 MΩ, as measured directly from the amplifier) was compensated by 60–70%. The mean input resistance was 254 ± 26 (SE) MΩ, and the mean resting potential was −69 ± 4 mV. The input resistance was monitored continuously, and the recording terminated if it varied by >10%. Field excitatory postsynaptic potentials (EPSPs) were recorded with the use of a low-resistance (1 MΩ) glass pipette placed in the medial perforant pathway.

Presynaptic stimulation was applied to the medial perforant pathway. Excitatory postsynaptic currents (EPSCs) and field EPSPs were recorded at a control frequency of 0.033 Hz. The amplitude of the test EPSP or EPSC was adjusted to one-third of maximum, ~1–1.5 mV for the field EPSPs and 50–100 pA for EPSCs. LTD was evoked by LFS consisting of 900 stimuli at 1 or 5 Hz, with the test stimulation voltage remaining at the same amplitude during the LFS. In the patch-clamp experiments, the LFS was applied under current-clamp conditions. LTP was induced by high-frequency stimulation (HFS) consisting of eight trains each of eight pulses at 200 Hz with an intertrain interval of 2 s, and under current-clamp conditions for the duration of the HFS in patch-clamp experiments. Recordings were analyzed with the use of the Strathclyde electrophysiological software (Dr. J. Dempster). Values are the means ± SE, and Student’s t-test was used for statistical comparison.

RESULTS

Control induction of LTD

LTD was induced by two procedures. The first method, used to investigate LTD of field EPSPs, was the standard prolonged LFS of 900 pulses. Such LFS at 1 or 5 Hz induced short-term depression (STD) and LTD of field EPSPs that lasted >30 min (Fig. 1). The amplitude of STD, measured at 1–5 min poststimulation, and LTD, measured at 25–30 min poststimulation, was 38 ± 1% and 40 ± 1% of control, respectively, after 1-Hz stimulation (n = 5) (Fig. 1A) (P < 0.001), and 43 ± 2% and 36 ± 1% of control, respectively, after 5-Hz stimulation (n = 5) (P < 0.001).

The second procedure, used to investigate the induction of LTD of patch-clamped EPSCs, was an intracellular pairing procedure consisting of brief LFS and mild depolarization (Fig. 1B). The amplitude of the test EPSC before and after LTD induction was measured at a potential of −70 mV, and LTD was induced by a 60-pulse LFS of 1 Hz for 1 min applied at a potential at −50 or −40 mV. Such a pairing procedure induced a stable LTD lasting for >1 h at holding potentials of both −50 and −40 mV. The values of STD and LTD were 31 ± 2% and 37 ± 1%, respectively (n = 5) (P < 0.001) with the use of a holding potential of −40 mV.

Low Ca2+ reduces, but does not abolish, LTD induction

To determine the dependency of LTD induction of field EPSPs on the external Ca2+ concentration, 1-Hz LFS was given in 0.8 mM extracellular Ca2+, a concentration of Ca2+ that reduced the test field EPSP by 60–70%. LFS at 1 Hz (900 pulses) applied in this low-Ca2+ medium resulted in the induction of a significant STD and LTD, although the LTD was of reduced magnitude compared with control. The amplitude of STD and LTD was 33 ± 2% and 26 ± 1% at 1–5 and 25–30 min post-LFS, respectively (n = 8), both values significantly depressed from the pre-LFS test EPSP (P < 0.001). Such LTD, although of a relatively large magnitude, was significantly reduced compared with control (P < 0.01) (Fig. 2).

It can be seen from the data presented in Fig. 2 that the induction of LTD was less sensitive to a lowering of extracellular Ca2+ than the induction of LTP. Thus HFS, applied in low-Ca2+ medium 40 min after LFS, did not induce LTP, the EPSP measuring 101 ± 1% (n = 5) of the pre-HFS values at 25–30 min post-HFS, a value not significantly increased from pre-HFS levels (P > 0.005, n = 5). In a series of control experiments in control Ca2+ (2.0 mM), HFS induced LTD of field EPSPs of 152 ± 5%.

Effect of d-AP5 on LTD induction

To determine the dependency of LTD induction on activation of NMDARs, LFS was applied in the presence of the NMDAR antagonist d-AP5. The effect of d-AP5 was investigated on LTD induced both by prolonged LFS and by the intracellular pairing procedure of brief LFS and depolarization. d-AP5 (100 μM) was perfused for 45 min before 1-Hz LFS to ensure complete equilibration.

The induction of LTD of patch-clamp recording of EPSCs by the intracellular pairing procedure was not blocked by AP5. Thus LTD measured 33 ± 2% and 35 ± 1% at 1–5 and 25–30 min after 1-Hz stimulation (1 min) at −40 mV (n = 5), values not significantly different from control (Fig. 3A).

Prolonged LFS (900 pulses at 1 Hz) induced LTD of field EPSPs in the presence of d-AP5, although the amplitude of the LTD was reduced from control (Fig. 3B). Thus STD and LTD had values of 20 ± 2% and 18 ± 1% at 1–5 and 25–30 min post-LFS (n = 8), significant STD and LTD compared with the pre-LFS values (P < 0.001). However, the STD and LTD were significantly reduced from control (P < 0.001). The amplitude of the LTD induced in the presence of d-AP5 was more variable than control, and, as shown in Fig. 3B, it was as large in certain experiments as in control.
Induction of long-term depression (LTD) by low-frequency stimulation (LFS).

A: LTD of the field excitatory postsynaptic potential (EPSP) was induced by LFS of 900 pulses applied at 1 Hz. The mean LTD was 40% at 25–30 min poststimulation ($n = 5$).

B: LTD of the patch-clamped excitatory postsynaptic current (EPSC) was induced by LFS of 60 pulses at 1 Hz paired with a depolarization to −40 mV under voltage-clamp conditions. The mean LTD measured 37%.
The induction of LTD of the field EPSP was much more sensitive to a block of NMDARs than the induction of LTP. Figure 3A shows that the induction of LTP by HFS was completely blocked in the presence of d-AP5, with the EPSPs measuring 93 ± 1% of the pre-HFS value and no significant increase occurring (P > 0.001, n = 8).

The time course of the field EPSPs was strongly reduced in the presence of d-AP5 (compare traces of field EPSPs in Figs. 1A and 3B). The half-decay time of EPSPs in control and d-AP5 was 8.6 and 5.1 ms, respectively.

**Ni^{2+} strongly inhibits LTD induction**

The possible role of T-type voltage-gated calcium channels in the induction of LTD was investigated by applying 1-Hz LFS in the presence of the T-type voltage-gated channel blocker Ni^{2+} (50 μM). Perfusion of Ni^{2+} did not alter the amplitude of the test field EPSPs or the test EPSCs (Fig. 4A). The Ni^{2+} was perfused for ≈45 min before LFS. Ni^{2+} also did not alter the extent of the depression of the EPSPs and EPSCs during the 1-Hz LFS stimulation. In four experiments, the EPSC was reduced to 73 ± 4% and 70 ± 5% of baseline in control and Ni^{2+}, respectively, by the 60th pulse at 1 Hz (Fig. 4B).

Ni^{2+} was found to inhibit the induction of LTD of both field EPSPs and EPSCs by LFS. Figure 4C shows that after 1-Hz LFS the induction of LTD of field EPSPs was completely suppressed in the presence of Ni^{2+}, the EPSP measuring 101 ± 1% (n = 5) at 30 min post-LFS (P < 0.005, n = 5). Only a very brief STD lasting 2–3 min, with a maximal amplitude of 29 ± 4% of control, was induced after LFS in the presence of Ni^{2+}. Figure 4D shows that after 1-Hz stimulation for 1 min at −40 mV, the induction of LTD of EPSCs was strongly suppressed (Fig. 4D), LTD measuring 6 ± 1% (n = 5) at 30 min post-LFS (P < 0.001). Only an STD lasting 5–10 min with a maximal amplitude of 20 ± 4% was induced in the presence of Ni^{2+}.

**Nifedipine does not inhibit LTD induction**

The possible role of L-type voltage-gated Ca^{2+} channels in the induction of LTD was investigated by applying 1-Hz LFS in the presence of the L-type Ca^{2+} channel blocker nifedipine. Perfusion of nifedipine (10 μM) was not found to inhibit the amplitude of the test field EPSPs or EPSCs (Fig. 5A). Figure 5B shows that after perfusion of nifedipine for 45 min, STD and LTD of field EPSPs measured 42 ± 2% and 36 ± 1% (n = 5) at 1–5 and 25–30 min post-LFS, a significant LTD compared with pre-LFS values, and not significantly different from control LTD. Figure 5C shows that LTD of EPSCs was also not altered by nifedipine, STD and LTD measuring 33 ± 2% and 37 ± 2% at 1–5 and 30 min after 1-Hz stimulation at −40 mV. Nifedipine was not found to alter the extent of the depression of the amplitude of the EPSC evoked during LFS at 1 Hz, the EPSC amplitude being reduced to 73 ± 5% and 71 ± 4% (n = 4) of baseline in control and nifedipine, respectively, by the 60th pulse at 1 Hz.

**Thapsigargin strongly inhibits LTD induction**

The role of intracellular Ca^{2+} stores in the induction of LTD was investigated by applying LFS in the presence of thapsigargin, an agent known to deplete intracellular Ca^{2+}.
FIG. 3. Effect of d(-)-2-amino-5-phosphonopentanoic acid (d-AP5) on the induction of LTD. A: d-AP5 (100 μM) did not significantly inhibit the induction of LTD of patch-clamped EPSCs induced by pairing 1-Hz afferent stimulation (60 pulses) with depolarization to −40 mV under voltage-clamp conditions, the LTD measuring 35% (n = 5). B: high concentration of d-AP5 reduced, but did not abolish, the induction of LTD of field EPSPs. Filled circles: 1-Hz LFS applied in the presence of 100 μM d-AP5 induced a significant mean LTD of 18% (n = 8), a value significantly smaller than in control slices. The LTD in d-AP5 showed substantial variation. Open circles: 1 experiment in which the LTD was of large magnitude. The graph also shows that HFS in d-AP5 completely abolished induction of LTP.
stores. The induction of LTD of the field EPSP by 1-Hz LFS was blocked by thapsigargin, although a pronounced STD remained. Perfusion of thapsigargin (2 μM) did not significantly alter the amplitude of the test EPSP. LFS at 1 Hz induced STD in the presence of thapsigargin measuring 29 ± 2% (n = 4) of control at 1–5 min post-LFS. The EPSP returned to its control value (99%, n = 4) by 10 min after stimulation, with no LTD occurring, the EPSP measuring 100 ± 1% at 25 min post-LFS (Fig. 6). The induction of LTD of EPSPs by LFS of 5 Hz (900 pulses) was similarly inhibited by thapsigargin. After 5-Hz LFS, a large STD was induced measuring 72 ± 2% (n = 5) at 1–5 min post-LFS, but LTD was completely suppressed, the EPSP measuring 105 ± 4% of control (n = 5), at 25–30 min post-LFS.

**Ruthenium red strongly inhibits LTD induction**

The effects of ruthenium red were investigated by allowing it to diffuse intracellularly into the cell from a patch-clamp electrode. The effect of ruthenium red (20 μM) was investigated on LTD of EPSCs induced by LFS of 5 Hz for 3 min applied under current clamp conditions, rather than 1 Hz for 15 min, to monitor the post-LFS EPSC for a longer period. Ruthenium red was found to inhibit the induction of LTD. After the establishment of the whole cell patch-clamp mode, test EPSCs were monitored for 15 min to allow access of the ruthenium red to the synapses. The induction of LTD was inhibited in the presence of ruthenium red. In control slices, the amplitude of STD and LTD was 44 ± 3% and 49 ± 2% (n = 5) of control at 1–5 and 25–30 min, respectively, post-LFS, a significant STD and LTD (P < 0.001) (Fig. 7). In the presence of ruthenium red, STD and LTD measured 9 ± 1% and 1 ± 1% (n = 5) at 1–5 and 25–30 min post-LFS, significant inhibition of STD and LTD (Fig. 7).

Ruthenium red did not inhibit the induction of LTP, as shown in Fig. 8. In these experiments, 5-Hz LFS applied initially did not induce LTD at 5 min after stimulation, demonstrating that ruthenium red had diffused to the synapses. Application of HFS then resulted in LTP measuring 218 ± 8% (n = 6), a value actually significantly greater than that in control experiments (160 ± 6%).

**4-Chloro-3-ethylphenol strongly inhibits LTD induction**

4-Chloro-3-ethylphenol is an agent recently found to induce Ca²⁺ release from a thapsigargin-sensitive intracellular Ca²⁺ store (Larini et al. 1995). 4-Chloro-3-ethylphenol (300 μM) was found to inhibit the induction of LTD after 1- or 5-Hz stimulation. LFS at 1 Hz induced a large STD 10–15 min in duration and measuring 43 ± 2% at 1–5 min post-LFS. However, LTD was strongly inhibited, measuring 4 ± 1% at 25–30 min post-LFS (Fig. 9).

**DISCUSSION**

The present study presents evidence for the induction of an NMDAR-independent LTD that is dependent on Ca²⁺ influx via Ni²⁺-sensitive low-voltage-activated Ca²⁺ channels and also dependent on release of Ca²⁺ from thapsigargin- and ruthenium-red-sensitive intracellular Ca²⁺ stores.

Two procedures were used for the induction of LTD in the present experiments: a conventional one involving the
FIG. 4. (continued)
induction of LTD of field EPSPs by application of 900 pulses at 1 Hz, and a second novel pairing procedure involving the induction of LTD of patch-clamped EPSCs by application of very brief 1-Hz stimulation coupled with mild depolarization to −50 or −40 mV. The mild depolarization used in the pairing procedure would not be expected to activate high-threshold Ca²⁺ channels, such as L channels, but would be expected to activate low-threshold channels. Thus, although T-type Ca²⁺ currents are transient if evoked by a large depolarization, a sustained T current can be evoked by a mild depolarization to between about −60 to −40 mV, the inactivation and activation curves crossing over between these potentials (Fisher et al. 1990; Magee and Johnston 1995).

The present study clearly demonstrates that an influx of Ca²⁺ is essential for the induction of LTD, because lowering extracellular Ca²⁺ from the control level resulted in a significant reduction in the amplitude of LTD. However, LTD induction had a low sensitivity to a reduction of the extracellular concentration of Ca²⁺, because a relatively large LTD (26%) could still be induced even when extracellular Ca²⁺ was lowered to a low level (0.8 mM). It was notable that the induction of LTP was much more dependent on the concentration of extracellular Ca²⁺ than the induction of LTD, because LTP induction was completely abolished in the low-Ca²⁺ medium. Mulkey and Malenka (1992) have previously shown that reducing extracellular Ca²⁺ from 2.5 to 0.5 mM resulted in a 20-Hz, 30-s tetanus inducing LTD expected to activate low-threshold channels. Thus, although T-type Ca²⁺ currents are transient if evoked by a large depolarization, a sustained T current can be evoked by a mild depolarization to between about −60 to −40 mV, the inactivation and activation curves crossing over between these potentials (Fisher et al. 1990; Magee and Johnston 1995).

An alternative hypothesis, which we favor on the basis of the results of the present experiments, is that whereas LTP induction depends on a large influx of Ca²⁺ from the extracellular medium, LTD induction depends on a relatively small initial influx of Ca²⁺ (a trigger Ca²⁺) followed by a large release of Ca²⁺ from intracellular stores by Ca²⁺-induced Ca²⁺ release at the ryanodine receptor (see below). The actual rise in intracellular Ca²⁺ may then be similar for
FIG. 5. Nifedipine (10 μM) does not inhibit the induction of LTD of field EPSPs or EPSCs. A: example of experiments in which perfusion of nifedipine did not alter the amplitude of the test field EPSP (○) or the test EPSC (●). B: 1-Hz LFS applied in the presence of nifedipine induced a mean LTD of the field EPSP of 36% (n = 5), a value not significantly different from control. C: 1-Hz LFS applied in the presence of nifedipine induced LTD of the EPSC of 37% (n = 5), a value not significantly different from control.
both the induction of LTP and LTD, which would be in agreement with recent experimental findings (Neveu and Zucker 1996).

The inhibition of LTD of both field EPSPs and EPSCs by low concentrations of Ni\(^{2+}\) strongly indicates that the LTD induction is dependent on Ca\(^{2+}\) influx via low-threshold voltage-gated Ca\(^{2+}\) channels. Ni\(^{2+}\) has previously been shown to block T-type Ca\(^{2+}\) channels with a median inhibiting concentration of ~50 \(\mu\)M (Bean 1989; Fox et al. 1987), including those located on hippocampal dendrites (Magee and Johnston 1995). Evidence for the presence of Ni\(^{2+}\)-sensitive T-type Ca\(^{2+}\) channels in hippocampal dendrites has been previously demonstrated with single-channel recordings (Magee and Johnston 1995), whereas subthreshold trains of synaptic potentials have also been shown to produce a Ni\(^{2+}\)-sensitive local increase in intradendritic Ca\(^{2+}\) concentration (Magee et al. 1995).

The induction of LTD of field EPSPs or EPSCs was not blocked in the present studies by a high concentration (10 \(\mu\)M) of nifedipine, a well-documented L-type voltage-gated Ca\(^{2+}\) channel blocker (Bean 1989), thus demonstrating that high-threshold L-type channels are not involved in the induction of LTD under the present protocols. Such a finding supports those of several previous studies in which L-type Ca\(^{2+}\) channel blockers have not been found to inhibit LTD induction induced by LFS (Bashir and Collingridge 1994; Mulkey and Malenka 1992; Selig et al. 1995). One study has presented pharmacological evidence for an involvement of L-type Ca\(^{2+}\) channels in the induction of LTD, although that study was in very young animals in which LTP had not developed, possibly indicating a different LTD induction mechanism (Bolshakov and Siegelbaum 1994).

In a previous study we demonstrated that d-AP5, at a concentration of 50 \(\mu\)M and perfused for 30 min before LFS, did not inhibit the induction of LTD of field EPSPs (O’Mura et al. 1995a,b). In view of the controversy on the role of NMDARs in the induction of LTD (see INTRODUCTION), the involvement of NMDARs was reexamined with the use of a high concentration of d-AP5 (100 \(\mu\)M), which we found in previous studies to completely inhibit NMDAR-mediated EPSCs (O’Connor et al. 1995). d-AP5 did not inhibit the induction of LTD when the pairing procedure of brief 1-Hz afferent stimulation coupled with depolarization to ~40 mV was used. Moreover, although the induction of LTD of field EPSPs was partially reduced by d-AP5, a substantial induction of LTD remained. These experiments demonstrate that calcium influx via NMDARs is not required for the induction of LTD in the dentate gyrus. Because activation of NMDARs contributes strongly to the EPSPs, the reduction of LTD induction of field EPSPs by AP5 observed in the present and previous studies (Debanne et al. 1994; Dudek and Bear 1992; Fuji et al. 1991; Mulkey and Malenka 1992; O’Dell et al. 1994; Selig et al. 1995; Thiels et al. 1994; Xiao et al. 1995) is most likely to be caused by the AP5-induced reduction of the amplitude/duration of EPSPs, thereby reducing activation of T-type voltage-gated calcium channels during LFS.

The present experiments provide evidence that the induction of LTD is dependent on functional intracellular ryanodine-receptor-sensitive Ca\(^{2+}\) stores. Thus LTD induction was prevented after depletion of intracellular Ca\(^{2+}\) stores by thapsigargin. A role for intracellular Ca\(^{2+}\) stores in the induction of LTD was further supported by the ability of ruthenium red to block LTD induction. Ruthenium red has been shown in previous studies to prevent the release of Ca\(^{2+}\) from intracellular stores by binding
FIG. 6. Thapsigargin strongly inhibits the induction of LTD of field EPSPs. The graph shows that 1-Hz LFS applied in the presence of thapsigargin did not result in the induction of significant LTD; mean of 5 experiments.

FIG. 7. Ruthenium red strongly inhibits the induction of LTD of intracellular EPSCs. The graph shows that in control slices (●), LFS of 5 Hz (900 pulses) applied 15 min after formation of the whole cell patch-clamp induced LTD of 49%; mean of 5 slices. In the presence of ruthenium red, applied in the patch-clamp electrode, LFS did not induce significant LTD. a and c: records of test EPSCs in the presence of ruthenium red and in control, respectively, before LFS. b and d: records of EPSCs following LFS in the presence of ruthenium red and in control, respectively.
FIG. 8. Ruthenium red does not block the induction of LTD of intracellular EPSCs. The graph shows that 5-Hz LFS applied 15 min after formation of the whole cell patch clamp did not induce LTD. After a further 5 min, application of HFS induced LTP measuring 218%; mean of 6 slices.

FIG. 9. 4-Chloro-3-ethylphenol blocks the induction of LTD of field EPSPs. The graph shows that 1-Hz LFS induced an STD lasting ~15 min, but no significant LTD; mean of 6 slices.
to the ryanodine receptor on intracellular Ca$^{2+}$ stores and preventing the opening of the Ca$^{2+}$ channel associated with the ryanodine receptor (Ma et al. 1988; Rousseau et al. 1987; Smith et al. 1988). Thus these experiments strongly suggest that Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores via the ryanodine receptor Ca$^{2+}$ channel is essential for the induction of LTD, supporting previous studies from this laboratory showing that dantrolene inhibited the induction of LTD (O’Mara et al. 1995a,b). The induction of LTD in the cerebellum was also inhibited by ruthenium red and thapsigargin, from which it was concluded that Ca$^{2+}$ release from internal ryanodine-receptor-sensitive Ca$^{2+}$ stores is required for the induction of LTD (Kohda et al. 1995).

Ruthenium red was not found to inhibit the induction of LTD, even at a time after its application at which the induction of LTD was fully blocked (thus showing that the ruthenium red had diffused to the synapses). This suggests that the induction of LTD is not dependent on Ca$^{2+}$ release from intracellular stores, suggesting strongly that the ryanodine-receptor-sensitive intracellular Ca$^{2+}$ stores may be required as a sink for Ca$^{2+}$ during the induction of LTD.

The results presented in this study are therefore consistent with the theory that LFS causes a relatively small influx of Ca$^{2+}$ via T-type voltage-gated Ca$^{2+}$ channels. Such Ca$^{2+}$ influx acts as a trigger Ca$^{2+}$, causing Ca$^{2+}$-induced Ca$^{2+}$ release from intracellular stores via the ryanodine receptor. Studies from this laboratory have previously shown that the induction of LTD at in the dentate gyrus requires the activation of metabotropic glutamate receptors (O’Mara et al. 1995b), activation of which is known to result in Ca$^{2+}$ release from intracellular stores. It is therefore postulated that simultaneous activation of T-type Ca$^{2+}$ channels and metabotropic glutamate receptors may result in “coincidence detection” in the process of induction of LTD, with Ca$^{2+}$ release from intracellular stores greatly potentiated when the T-type Ca$^{2+}$ channels and metabotropic glutamate receptors are activated together.

This work was supported by the Health Research Board, Ireland, the Wellcome Trust and the E.U. Address for reprint requests: Y. Wang, Dept. of Physiology, Trinity College, Dublin 2, Ireland.

Received 12 March 1996; accepted in final form 7 October 1996.

REFERENCES


NEVUE, D. AND ZUCKER, R. S. Postsynaptic levels of calcium needed to trigger LTD and LTP. Neuron 16: 619–629, 1996.


THELS, E., BARIONUEVO, G., AND BERGER, T. W. Excitatory stimulation...

