LTP Induction Dependent on Activation of Ni\(^{2+}\)-Sensitive Voltage-Gated Calcium Channels, but not NMDA Receptors, in the Rat Dentate Gyrus In Vitro

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Wang, Yue, Michael J. Rowan, and Roger Anwyl. LTP induction dependent on activation of Ni\(^{2+}\)-sensitive voltage-gated calcium channels, but not NMDA receptors, in the rat dentate gyrus in vitro. J. Neurophysiol. 78: 2574–2581, 1997. A N-methyl-D-aspartate receptor (NMDAR)-independent long-term potentiation (LTP) has been investigated in the dentate gyrus of the hippocampus in vitro in the presence of the NMDAR antagonist, d-2-amino-phosphonopentanoate (50–100 \(\mu\)M), at a concentration that completely blocked NMDAR-mediated excitatory postsynaptic currents (EPSCs). LTP of patch-clamped EPSCs was induced by pairing low-frequency evoked EPSCs (1 Hz) with depolarizing voltage pulses designed to predominantly open low-voltage–activated (LVA) Ca\(^{2+}\) channels. Voltage pulses alone induced only a short-term potentiation. The LTP was blocked by intracellular application of the rapid Ca\(^{2+}\) chelator bis-(\(\alpha\)-aminophenoxy)-N,N,N’,N’-tetraacetic acid, demonstrating that a rise in intracellular Ca\(^{2+}\) is required for the NMDAR-independent LTP induction. The NMDAR-independent LTP induction also was blocked by Ni\(^{2+}\) at a low extracellular concentration (50 \(\mu\)M), which is known to strongly block LVA Ca\(^{2+}\) channels. However, Ni\(^{2+}\) did not inhibit the NMDAR-dependent LTP induced by high-frequency stimulation (HFS). The NMDAR-independent LTP induction was not blocked by high concentrations of the L-type Ca\(^{2+}\) channel blocker nifedipine (10 \(\mu\)M). The NMDAR-independent LTP was inhibited by the metabotropic glutamate receptor ligand (\(+\))-\(\alpha\)-methyl-4-carboxyphenylglycine. These experiments demonstrate the presence of a NMDAR-independent LTP induced by Ca\(^{2+}\) influx via Ni\(^{2+}\)-sensitive, nifedipine-insensitive voltage-gated Ca\(^{2+}\) channels, probably LVA Ca\(^{2+}\) channels. Induction of the NMDAR-independent LTP was inhibited by prior induction of HFS-induced NMDAR-dependent LTP, demonstrating that although the NMDAR-dependent and NMDAR-independent LTP use a different Ca\(^{2+}\) channel for Ca\(^{2+}\) influx, they share a common intracellular pathway.

INTRODUCTION

Long-term potentiation (LTP) is a long-lasting and stable increase in excitatory synaptic transmission induced by co-activation of both presynaptic and postsynaptic cells. The induction of LTP is known to be dependent on a rise in the intracellular Ca\(^{2+}\) concentration. Thus loading the postsynaptic cell with intracellular Ca\(^{2+}\) chelators was found to inhibit the induction of LTP ( Lynch et al. 1983; Malenka et al. 1988; Komatsu et al. 1991). However, the intracellular release of caged Ca\(^{2+}\) led to the induction of LTP (Malenka et al. 1988). The source of the Ca\(^{2+}\) responsible for LTP induction is known to be, at least in part, extracellular, as decreasing external Ca\(^{2+}\) reduced the induction of LTP (Dunwiddie and Lynch 1979; Mulkeen et al. 1988). The Ca\(^{2+}\) permeable N-methyl-D-aspartate receptor (NMDAR) (MacDermott et al. 1986) is known to be one type of channel by which Ca\(^{2+}\) entering the cell leads to the induction of LTP (Collingridge et al. 1983; Harris et al. 1986). Such NMDAR-dependent induction of LTP has been thought to be the prevalent form of LTP induction at synapses at which NMDARs are located, such as the CA1 (Collingridge et al. 1983), the dentate gyrus (Morris et al. 1986), and the neocortex (Artola and Singer 1987).

NMDAR-independent LTP induction also has been described previously, although much less commonly than NMDAR-dependent LTP. Thus NMDAR-independent Ca\(^{2+}\) influx via nifedipine-sensitive, L-type high-voltage–activated (HVA) voltage-gated Ca\(^{2+}\) channels has been shown to induce a NMDAR-independent LTP after very high-frequency stimulation (HFS) (200 Hz) in CA1 hippocampus (Grover and Teyler 1990). However, such L-type Ca\(^{2+}\) channel-dependent form of LTP differed from NMDAR-dependent LTP in having a very slow (10–15 min) rise time (Grover and Teyler 1990). Moreover, the L-type Ca\(^{2+}\) channel-dependent LTP was a relatively small component of the total HFS-induced LTP (Grover and Teyler 1990).

Kullmann et al. (1992) also have reported briefly the facilitation of induction of a low-frequency–induced (LFS) NMDAR-independent LTP in CA1 in high Ca\(^{2+}\) media by pairing the LFS with depolarizing pulses. In addition, a form of LTP in CA1 produced by the K\(^{+}\) channel blocker tetraethylammonium was shown to be NMDAR-independent (Aniksztejn and Ben-Ari 1991). NMDAR-independent LTP induction also has been found in other regions, notably the dorsolateral septal nucleus, where HFS induced a NMDAR-independent LTP (Zheng and Gallagher 1992a), and the visual cortex, where a NMDAR-independent LTP was induced by LFS (Komatsu et al. 1991).

In the present experiments, we show that a NMDAR-independent LTP in the rat dentate gyrus is induced by a protocol involving the pairing of excitatory postsynaptic currents (EPSCs) with voltage pulses designed to activate predominately low-voltage–gated, Ni\(^{2+}\)-sensitive Ca\(^{2+}\) channels.

METHODS

Standards methods were used to make extra- and intracellular recordings from the dentate gyrus of Wistar rats, as described previously (O’Connor et al. 1995; Wang et al. 1996). Trans-
verse slices (350 μm thick) of the rat hippocampus (weight 40–80 g) were prepared using standard techniques. They were superfused continuously at a rate of 8 ml/min at 30–32°C in a media containing (in mM) 120 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2.0 MgSO₄, 2.0 CaCl₂, and 10 d-glucose, bubbled with 95% O₂-5% CO₂. All solutions contained 100 μM picrotoxin (Sigma) to block γ-aminobutyric acid-Å-mediated activity. Additional drugs used were d-2-amino-phosphopentanoate (d-AP5, Tocris Cookson), (+)-α-methyl-4-carboxyphenylglycine ( MCPG , Tocris Cookson), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoline (NBQX, Tocris), and nifedipine (Sigma). The latter was applied in 0.02% dimethyl sulfoxide with precautions taken to prevent light inactivation (perfusion system covered in dark foil, microscope light turned off, room darkened). The patch-clamp electrode, resistance 5–8 MΩ, contained (in mM) 130 potassium gluconate, 10 KCl, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′′-tetraacetic acid (EGTA), 1 CaCl₂, 3 MgCl₂, 20 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 5 MgATP, and 0.5 NaGTP. pH 7.2 (using KOH). Such an intracellular medium has been calculated to give a free internal Ca²⁺ concentration of 17.5 nM. In experiments with the rapid Ca²⁺ chelator bis-(o-aminophenoxy)-N,N,N′,N′′-tetraacetic acid (BAPTA; 20 mM), potassium gluconate was reduced to 120 mM and EGTA omitted.

EPSCs were recorded in the whole cell patch-clamp mode from dentate granule cells using an Axopatch 1D amplifier (3 kHz low-pass Bessel filter). The capacitive current was cancelled electronically and the series resistance (6–20 MΩ as measured directly from the amplifier) compensated by ~60–70%. The mean input resistance was 283 ± 24 (SE) MΩ, and the mean resting potential -72 ± 4 mV. The input resistance was monitored continuously, and the recording terminated if it varied by >10%. Recordings of the amplitude of field excitatory postsynaptic potentials (f-EPSPs) were made using a low-resistance (~1 MΩ) glass pipette placed in the medial perforant pathway. EPSCs and f-EPSPs were recorded in response to stimulation of the medial perforant pathway at a control frequency of 0.033 Hz with the stimulation intensity adjusted to evoke an EPSC that was ~30% of the maximum amplitude, usually ~50–75 pA for α-amino-3-hydroxy-5-methyl-4-isoxazolopionic acid (AMPA) EPSCs, and 1.2 mV for f-EPSPs. The cells always were held at a potential of ~70 mV for measurement of test EPSCs. LTP of f-EPSPs was induced by HFS consisting of eight trains each of eight pulses at 200 Hz, intertrain interval 2 s. Full experiments were carried out providing that certain criteria were met. These included a resting potential of at least ~65 mV, a high-input resistance (at least 200 MΩ), and a low threshold and steep input-output curve for the EPSCs.

Recordings were analyzed using the Strathclyde electrophysiological software (Dr. J. Dempster). Values are the means ± SE, and Student’s t-test was used for statistical comparisons.

**RESULTS**

Low-frequency EPSCs paired with depolarizing voltage pulses induces a NMDAR-independent LTP

All experiments were carried out in the presence of the NMDAR antagonist d-AP5 (50–100 μM) to completely block activation of NMDAR, and cells were measured at -70 mV under whole cell clamp conditions while measuring test EPSCs.

The voltage pulse protocol consisted of application of a 500-ms prepulse to -100 mV, designed to fully remove any inactivation of voltage-gated Ca²⁺ channels, followed by a 500-ms depolarizing pulse to 0 mV to strongly activate the Ca²⁺ channels. A single EPSC was evoked during each depolarizing pulse. A 1-Hz pairing of EPSCs and depolarizing pulses was very effective at inducing LTP. Five such consecutive pairings induced LTP measuring 160 ± 4%, n = 18, at 30 min postpairing (P < 0.001; Fig. 1A). Even two pairings induced significant LTP of 128 ± 4%, n = 5 (P < 0.01) and 17 pairings induced LTP of 189 ± 5% (Fig. 1B).

Five pairings of EPSCs and pulses from -100 to 0 mV were used as the standard protocol in this study. However, a number of experiments were carried out in which pairings of EPSCs were made with different voltage pulse protocols. In a set of experiments designed to open LVA rather than HVA Ca²⁺ channels, EPSCs were paired with mild depolarizing pulses. Thus the potential was prepulsed to -100 mV and then pulsed to -50 mV. Five such pairings induced a small but significant LTP measuring 119 ± 7% (P < 0.05, n = 10), whereas 10 such subsequent further pairings induced LTP of 130 ± 6% (Fig. 1C). An additional set of experiments involved prepulsing to only ~75 mV before depolarization of pulses to 0 mV. Although such a protocol would be expected to remove inactivation of Ca²⁺ channels only partially, the change in potentials involved would approximate more closely to those occurring physiologically. Five such pairings were found to induce LTP of 131 ± 3%, P < 0.05, n = 5 (Fig. 1D). This was a significantly smaller LTP than that induced by the prepulsing to ~100 mV (P < 0.05).

A further set of experiments was designed to open preferentially HVA Ca²⁺ channels by holding the potential at a relative depolarized potential prior to depolarization (~55 mV) and applying large-amplitude voltage pulses (to +20 mV). Such a protocol would also be expected to largely inactivate LVA channels. Five EPSCs paired with pulses from -55 to +20 mV did not induce significant LTP (97 ± 4%, P > 0.05, n = 6; Fig. 1E).

A complete block of the NMDAR-mediated EPSCs was verified by the demonstration that the EPSCs evoked in 50 μM d-AP5 were pure AMPA receptor (AMPA)-mediated EPSCs, with no NMDAR-mediated component. Perfusion of NBQX (2 μM), a highly selective AMPAR antagonist (Sheardown et al. 1990), in the presence of 50 μM d-AP5 completely abolished the EPSCs recorded at -70, -30, +30, and +60 mV, n = 5, with no NMDAR-mediated EPSC remaining (Fig. 2). Even increasing the afferent stimulation voltage to a much higher level than normally used did not evoke an EPSC in the presence of both NBQX and d-AP5.

Depolarizing pulses applied without pairing with EPSCs induced STP and not LTP

To determine whether Ca²⁺ influx via voltage-gated Ca²⁺ channels alone could induce LTP, the hyperpolarizing-depolarizing voltage pulses (~100 to 0 mV) were applied without pairing with the EPSCs. A short-term potentiation (STP) lasting ~10 min and with a peak amplitude of 153 ± 8%, n = 6, at 1 min postpulsing was induced by five voltage pulses alone, but LTP was not induced (EPSCs measured 101 ± 4%, n = 6, P > 0.05 at 30 min postpulsing; Fig. 3, A and B). Figure 3B also shows that after the induction of STP by pulses alone, the application of pairing of pulses with EPSCs at a short time after the STP was able to induce LTP (n = 3). Twenty voltage pulses from -100 to 0 mV...
also were found to induce only STP (136 ± 2%, n = 4 at 1 min postpulsing) but not LTP (101 ± 2% at 30 min postpulsing).

**Intracellular application of a Ca\(^{2+}\) chelator blocks induction of NMDAR-independent LTP induction**

Evidence that the NMDAR-independent LTP was induced by a rise in intracellular Ca\(^{2+}\) was established in experiments in which intracellular application of the rapid Ca\(^{2+}\) chelator BAPTA (20 mM) was found to completely block the induction of LTP after the pairing of five pulses from −100 to 0 mV with EPSCs, the test EPSCs measuring 100 ± 4% and 95 ± 4% (P > 0.05, n = 5) at 10 and 40 min postpairing (Fig. 4).

\(\text{Ni}^{2+}\), but not nifedipine, inhibits the induction of the NMDAR-independent LTP

\(\text{Ni}^{2+}\) (50 μM) blocked the induction of the pairing induced NMDAR-independent LTP. \(\text{Ni}^{2+}\) was perfused for 45 min before applying the pairing. Previous studies by the present authors have shown that this concentration of \(\text{Ni}^{2+}\) did not reduce the amplitude of EPSCs evoked at the test frequency (Wang et al. 1997a). Figure 5A shows that perfusion of \(\text{Ni}^{2+}\) completely blocked the induction of the LTP.
by the pairing of five EPSCs with voltage pulses from -100 to 0 mV, the test EPSCs measuring 100 ± 1 and 93 ± 5% at 10 and 20 min postpairing ($P < 0.001, n = 5$).

It has been reported previously that Ni$^{2+}$ (25 μM) inhibited NMDAR-dependent LTP in CA1 (Ito et al. 1995). However, Ni$^{2+}$ was not found to inhibit NMDAR-dependent LTP of EPSCs induced by HFS in the present study in the dentate gyrus. Thus after perfusion of Ni$^{2+}$ for 45 min, LTP induced by HFS (8 trains of 8 pulses at 200 Hz, intertrain interval 2 s) at a steady state holding potential of 0 mV measured 153 ± 9%, $n = 5$, at 30 min post-HFS. This was not significantly different from the control HFS-induced LTP, which measured 151 ± 7%, $n = 5$, $P > 0.05$, at 30 min post-HFS (Fig. 5B).

Nifedipine (10 μM) did not inhibit the induction of the NMDAR-independent LTP. Figure 5C shows that LTP measuring 147 ± 4% was induced after the pairing of five EPSCs with pulses from -100 to 0 mV in the presence of 10 μM nifedipine ($P > 0.005$, $n = 5$).

**NMDAR-independent LTP is blocked by MCPG**

The involvement of metabotropic glutamate receptor (mGluR) in the induction of the NMDAR-independent LTP was investigated by pairing the voltage pulses with EPSCs in the presence of the mGluR ligand (+) MCPG (500 μM). The induction of LTP was blocked completely by MCPG, the EPSCs measuring 101 ± 4% at 20 min postpairing ($P < 0.001, n = 5$), although an STP lasting ~10 min and with a peak amplitude measuring 118 ± 3%, $n = 5$, always remained in the presence of MCPG (Fig. 6).

**Induction of NMDAR-dependent LTP occludes the induction of NMDAR-independent LTP**

Occlusion experiments were carried out to determine if the voltage-gated Ca$^{2+}$ channel-dependent LTP could be induced after maximal induction of the NMDAR-dependent LTP. A two-pathway stimulation protocol was used in these experiments. Two series of HFS separated by a 10-min interval were applied to one stimulation pathway (pathway 1, Fig. 7) to saturate LTP in this pathway, LTP of f-EPSPs being monitored with an extracellular electrode. Whole cell patch-clamp recordings of EPSCs then were made from a cell in response to stimulation of pathway 1 and a second independent pathway (pathway 2, Fig. 7). Pairing of pulses from -100 to 0 mV with EPSCs evoked from pathway 1 in which LTP had been saturated did not induce Ca$^{2+}$ channel-dependent LTP, the EPSCs measuring 101 ± 2%, $n = 9$, at 10 min postpairing ($P > 0.05$). However, Ca$^{2+}$ channel-dependent LTP was induced (135 ± 6%, $P < 0.001, n = 9$) after application of the pairing protocol to the second pathway, which previously had received only test stimulation.

**DISCUSSION**

The present study shows that a NMDAR-independent LTP can be induced in the dentate gyrus by a pairing procedure...
consisting of low-frequency-evoked EPSCs and depolarizing voltage pulses. Activation of NMDAR was inhibited by inclusion of 50–100 μM of the NMDAR antagonist D-AP5 in the perfusing media. This high concentration of D-AP5 was shown to completely block activation of NMDAR, as no NMDAR-mediated EPSC remained in the presence of the AMPA antagonist NBQX at a range of holding potentials. Moreover, recent studies from this laboratory have shown that isolated NMDAR-mediated EPSCs recorded from dentate granule cells are completely abolished by 50 μM D-AP5 (O’Connor et al. 1995) in agreement with studies in CA1 (Bashir et al. 1991; Kullmann et al. 1992). Moreover, D-AP5 at concentrations of 25–50 μM completely blocked NMDAR-dependent LTP induced by both a tetanic stimulation (Collingridge and Bliss 1987; Malenka 1991; Zalutsky and Nicol 1990) and by the pairing procedure of prolonged intracellular depolarization and low-frequency synaptic stimulation (Kullmann et al. 1992). A very small NMDAR-mediated EPSP was found in 50 μM D-AP5 with strong afferent stimulation in the study of Hanse and Gustafsson (1995) in CA1, the summation of which led to the induction of appreciable LTP after repeated strong tetani although not a single strong tetanus. In the present study, we present strong evidence that the LTP induced in the presence of 50–100 μM D-AP5 was not generated by a small remaining NMDAR-mediated EPSP. First, no such NMDAR-mediated component of the EPSC remained in 50 μM D-AP5 perhaps because of the lower density of NMDAR in the dentate gyrus compared with CA1 (Monaghan and Cotman 1983). Second, 50 μM D-AP5 was sufficient to completely block HFS-induced LTP at a holding potential of 0 mV in our studies, a stimulation protocol that would result in a much more powerful NMDAR activation than the 1 Hz stimulation used in the present study (Wang et al. 1997). Third, Ni²⁺ did not block the NMDAR-dependent LTP but did block the NMDAR-independent LTP.

The experiments detailed in this study demonstrate that the NMDAR-independent LTP at the medial perforant pathway-granule cell synapse is induced by an increase in the intracellular Ca²⁺ concentration, as strong chelation of intracellular Ca²⁺ prevented the LTP induction. Such an increase in Ca²⁺ could be produced by the influx of Ca²⁺ via voltage-gated Ca²⁺ channels, by the release of Ca²⁺ from intracellular stores (Zheng and Gallagher 1992b; Zheng et al. 1996), by the activation of the Ca²⁺-activated nonspecific cationic current, CAN (Crepel et al. 1994), or by the activation of a Na⁺/Ca²⁺ exchange mechanism (Knopfel et al. 1991). The experiments in the present study using different pulse protocols and also pharmacological blockers of voltage-gated Ca²⁺ channels strongly suggest that Ca²⁺ influx via voltage-gated channels,
and in particular, LVA channels, is the most likely mechanism by which intracellular Ca\textsuperscript{2+} is increased.

A large number of studies have shown the presence of both LVA and HVA voltage-gated Ca\textsuperscript{2+} channels on granule cells in the dentate gyrus (Blaxter et al. 1989; Eliot and Johnston 1994; Fisher et al. 1990), on pyramidal cells of the hippocampus (Avery and Johnston 1996; Docherty and Brown 1986; Fisher et al. 1990; Frazer and MacVicar 1991; O’Dell and Alger 1991; Magee and Johnston 1995), and on cells in many other regions of the brain (Bean 1989; Tsien et al. 1988). Furthermore, LVA channels predominate in hippocampal dentrites (Kavalali et al. 1997; Magee and Johnston 1995), which would result in the dominance of LVA channels in dendritic Ca\textsuperscript{2+} influx (Kavalali et al. 1997). Moreover, subthreshold EPSPs have been shown in previous studies to activate LVA voltage-gated Ca\textsuperscript{2+} channels in dendrites of the hippocampus and cortex and result in localized increases in intradendritic Ca\textsuperscript{2+} (Magee et al. 1995; Markram and Sakmann 1994). The NMDAR-independent LTP induced by pairing of EPSCs with different pulse protocols in the present study indicates that the LTP is induced predominately by Ca\textsuperscript{2+} influx via LVA Ca\textsuperscript{2+} channels. The standard voltage pulse protocol used in the present study (prepulse to -100 mV followed by depolarization to 0 mV) would activate strongly both LVA and HVA channels (Kavalali et al. 1997). The induction of the NMDAR-independent LTP by Ca\textsuperscript{2+} influx via LVA Ca\textsuperscript{2+} channels in the present study also is supported by the block of the LTP by a relatively low concentration of Ni\textsuperscript{2+} (50 \mu M). Ni\textsuperscript{2+} at similar low concentrations has been shown previously in numerous studies to inhibit preferentially transient, LVA Ca\textsuperscript{2+} channels in granule cells of the dentate gyrus (Blaxter et al. 1989; Eliot and Johnston 1994) and other cells of the brain (Avery and Johnston 1996; Bean 1989; Fox et al. 1987; Zhang et al. 1993). However, similar low concentrations of Ni\textsuperscript{2+} also have been shown to inhibit the R-type Ca\textsuperscript{2+} channels in CA1 neurons (Magee and Johnston 1995) and in cerebellar cells (Zhang et al. 1993). Such R-type channels are transient Ca\textsuperscript{2+} channels with a voltage activation overlapping LVA/HVA. In CA3 pyramidal cells, 50 \mu M Ni\textsuperscript{2+} also slightly reduced nifedipine-sensitive sustained LVA and HVA Ca\textsuperscript{2+} currents, as well as reducing the transient LVA by about two-thirds (Avery and Johnston 1996). The block of the NMDAR-independent LTP induction by Ni\textsuperscript{2+} in the present studies is evidence for involvement of T-type or R-type LVA Ca\textsuperscript{2+} channels in the induction of the NMDAR-independent LTP. The only previous study suggesting the involvement of a Ni\textsuperscript{2+}-sensitive Ca\textsuperscript{2+} channel in the induction of LTP was the study of Komatsu and Iwakiri (1992) in the visual cortex, in which an NMDAR-independent LTP induced by prolonged LFS (2 Hz, 15 min) was blocked by 100 \mu M Ni\textsuperscript{2+}.

The inability of nifedipine to inhibit the induction of the NMDAR-independent LTP provides evidence that L-type Ca\textsuperscript{2+} channels are not involved in the induction of such LTP, as nifedipine and other related dihydropyridines have been shown in many studies to inhibit L-type Ca\textsuperscript{2+} channels in granule cells in the dentate gyrus (Blaxter et al. 1989; Eliot and Johnston 1994) and other neurons (Bean 1989; Docherty and Brown 1986; O’Dell and Alger 1991; Tsien et al. 1988). The concentration of nifedipine (10 \mu M) used in the present study is a saturating dose in the dentate gyrus for block of NMDAR-dependent LTP induced by HFS, as dihydropyridine L-type Ca\textsuperscript{2+} channel antagonists did not
inhibit such LTP (Huang and Malenka 1993; Kullman et al. 1992; Taube and Schwartzkroin 1986). LTP induced by Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels has been described previously (Grover and Tyler 1990), but such potentiation was of relatively small magnitude, induced only by very high-frequency stimulation and had a very slow rise time (10–15 min). In contrast, the potentiation described in the present study had a rapid rise time, within 1 min, similar to that of conventional NMDAR-dependent LTP.

We have shown in the present experiments that the pairing of voltage pulses with synaptic stimulation is an absolute requirement for LTP induction. The application of depolarizing pulses alone, in the absence of pairing with synaptic stimulation, only induced a STP and not LTP. This is in agreement with the study of Kullmann et al. (1992) in which depolarizing pulses from −90 to 0 mV induced only STP. Kullmann et al. (1992) were unable to demonstrate the induction of LTP in CA1 by the pairing of the depolarizing pulses with low frequency EPSCs in control media, although they did show that a large number of such pairs (150–200) produced an enhancement of the 2-Hz–induced control NMDAR-independent LTP in an elevated Ca\(^{2+}\) media. It is possible that the voltage-gated channels involved in the induction of NMDAR-independent LTP may be present at a lower density in CA1 than dentate gyrus, and thus the NMDAR-independent LTP only is induced in high Ca\(^{2+}\) media in CA1. NMDAR are known to be located at the highest density of any brain region in CA1 (Monaghan and Cotman 1983), and therefore NMDAR-dependent LTP might be expected to be the dominant form of LTP in this area.

The mGluR ligand MCPG was found to block the induction of the NMDAR-independent LTP produced by the pulse-EPSC pairing protocol in the present study. MCPG has been shown previously to inhibit LTP in certain previous studies (Bashir et al. 1993; Bortolotto et al. 1994; O’Connor et al. 1994; Wang et al. 1995). As such, mGluR may be important in LTP induction in the present studies through the activation of an intracellular messenger such as protein kinase C (Pin and Duvoisin 1995).

LVA Ca\(^{2+}\) channels are activated most strongly during low-frequency hyperpolarizing-depolarizing membrane sequences. These would occur, for example, when EPSPs follow either inhibitory postsynaptic potentials or spike-mediated afterhyperpolarizations and also during low-frequency rhythms, such as theta activity (4–8 Hz), δ-waves (1–4 Hz), and slower rhythms of 0.1–0.8 Hz (Bland 1990; Steriade 1993). The theta rhythm, during which the membrane potential undergoes oscillations of ±30 mV, and bursts of low-threshold Ca\(^{2+}\) spikes are generated on the depolarizing phase of the oscillations, is particularly prominent in the hippocampus, occurring during exploratory behavior and paradoxical phases of sleep (Bland 1990; Buzaki 1987). Thus LTP dependent on Ca\(^{2+}\) influx via voltage-gated Ca\(^{2+}\) channels may occur during such theta activity and underlie the cellular basis of memory formation during exploratory activity. A greatly sensitized LTP has been shown previously to occur at the peak of the theta rhythm both in vivo (Pavlides et al. 1988) and in vitro (Huerta and Lisman 1993; 1995).

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INDUCTION OF Ni²⁺-DEPENDENT NMDAR-INDEPENDENT LTP


