VDCCs and NMDARs Underlie Two Forms of LTP in CA1 Hippocampus In Vivo

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Morgan, S. L. and T. J. Teyler. VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. J. Neurophysiol. 82: 736–740, 1999. N-methyl-D-aspartate receptor/channel (NMDAR) and voltage-dependent calcium channel (VDCC) antagonists applied independently reduce the magnitude of long-term potentiation (LTP) in area CA1 of the hippocampal slice preparation. When used in combination, the antagonists completely block the induction of LTP. In urethan-anesthetized rats we examined the effect of the NMDAR blocker MK-801 (0.1 mg/kg) and the VDCC blocker Verapamil (10 mg/kg) on LTP induction in area CA1. Extracellular recordings were obtained from stratum radiatum following stimulation of Schaffer collaterals. LTP was induced by a 200-Hz/100-ms tetanus repeated 10 times (2 s isi). Tetanus was given in the presence of intraperitoneal saline, MK-801, Verapamil, or both Verapamil and MK-801. When given separately, Verapamil and MK-801 both significantly reduced the magnitude of LTP as compared with control animals. When given together, the drugs blocked the induction of LTP completely. We conclude that like LTP in vitro, VDCCs and NMDAR underlie two forms of LTP in vivo.

INTRODUCTION

Long-term potentiation (LTP) is an activity-dependent enduring increase in synaptic activity that is thought to be a cellular correlate of learning and memory. In the past, in an effort to understand the cellular/molecular characteristics of LTP, many investigators narrowed their focus to the study of influx of calcium through the N-methyl-D-aspartate receptor/channel (NMDAR) complex, as well as the downstream events that occurred due to calcium influx through this channel. NMDAR-dependent LTP (nmdaLTP) requires Ca²⁺ influx and cell depolarization, involves protein kinases, and displays input specificity (Dunwiddie and Lynch 1979; Kelso et al. 1986; Lynch et al. 1983; Malenka et al. 1988; Malinow and Miller 1986). Grover and Teyler (1990) demonstrated that LTP can be induced in CA1 hippocampal slices when NMDA channels were completely blocked with 2-amino-5-phosphonovaleric acid (APV) (Grover and Teyler 1994). This APV-resistant LTP could, however, be blocked with the L-type voltage-dependent calcium channel (VDCC) antagonist Nifedipine (Grover and Teyler 1990), showing that calcium via VDCCs was required for this form of LTP (vdccLTP). Bath application of the K⁺ channel blocker tetraethylammonium (TEA) onto hippocampal slices also revealed an NMDAR-independent form of LTP that was dependent on VDCCs (Aniksztejn and Ben-Ari 1991).

NmdaLTP and vdccLTP share some characteristics but are mechanically distinct from one another. Both types of LTP are blocked by calcium chelators, induced by tetanic afferent stimulation, and display input specificity (Grover and Teyler 1990, 1992; Kato et al. 1993). VdccLTP can be blocked completely by the application of L-type (dihydropyridine sensitive) calcium channel blockers and tyrosine kinase inhibitors, whereas nmdaLTP is unaffected. Conversely, serine/threonine kinase inhibitors have no effect on vdccLTP, while blocking nmdaLTP completely (Cavus and Teyler 1996). The different phosphorylation cascades initiated in the two forms of LTP may result in differential regulation of gene expression, which could indicate two different functions of these forms of plasticity (Bading et al. 1993; Cavus and Teyler 1996; Ghosh and Greenberg 1995).

In this experiment, we induced LTP in CA1 hippocampus in an intact anesthetized preparation, using stimulation parameters known to induce two forms of LTP in slices. Using a single tetanus parameter and separating the two forms of LTP pharmacologically with NMDAR and VDCC antagonists, we found evidence for both vdccLTP and nmdaLTP in this preparation.

METHODS

Male Long Evans rats (50–70 days old) were surgically prepared for acute recordings. Animals were anesthetized with 1.5 g/kg urethan and placed in a stereotactic device for surgery and recording. Heating pads and a heating lamp were used to maintain the temperature of the animals at 37.5 ± 0.5°C. Burr holes were drilled in the skull above the final recording and stimulating sites. The recording electrode was placed in the stratum radiatum area of CA1 (3.8 posterior to bregma, 2.0 lateral to bregma). The monopolar stimulating electrode was placed in the Schaffer collateral/commissural pathway distal to the recording electrode (3.8 posterior, 2.8 lateral, depth 2.8–3.4). All electrodes were constructed from 000 insect pins insulated with Epoxytite except at the tips. Electrodes were lowered slowly through the cortex until the hippocampus was achieved as determined by physiological and stereotaxic indicators. We allowed 60 min to elapse before recording to allow for a period of stabilization. Test stimuli were delivered to the Schaffer collateral/commissural pathway every 30 s. Electrode depth was then adjusted to elicit a maximal evoked field potential. The test intensity was adjusted to elicit a field excitatory postsynaptic potential (fEPSP) that was ~2.8 mV, which corresponded to ~35% of the maximal fEPSP, for each preparation. LTP was induced with a tetanus of 200 Hz/100 ms every 2 s, repeated 10 times, at a stimulation intensity that elicited an fEPSP of 35% of the maximal fEPSP. Pharmacological manipulation was used to isolate nmdaLTP from vdccLTP. NMDARs were blocked using Dizocilpine Maleate (+MK-801, 0.1 mg/kg), and VDCCs were blocked with Verapamil Hydrochloride (±Verapamil, 10 mg/kg). Drugs were injected intraperitoneally 1 h before tetanus, and neither drug had any effect on baseline responses. Control animals...
were injected intraperitoneally with physiological saline. Extracellular potentials were amplified (x100), band-pass filtered (0.01 Hz to 3,000 kHz), and digitized for later analysis. A running average of three fEPSPs was taken for graphic presentation and data analysis. Changes in the fEPSP were expressed as a percentage change in initial slope, relative to the mean initial slope in the baseline period. Student’s t-tests were used to determine the significance of observed differences between the forms of LTP. The point of significant difference was set to $P < 0.05$.

Stock solutions were prepared fresh weekly and stored at $-20\,^\circ\mathrm{C}$ when not in use. Solutions were never used more than 1 wk. Verapamil and MK-801 were prepared to optimal concentration in distilled water. Precautions were taken with light-sensitive drugs to ensure their potency.

**RESULTS**

In the absence of either drug, the tetanus induced LTP, as measured by an increase in initial slope averaged across the

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**FIG. 1.** Representative field excitatory postsynaptic potentials (fEPSPs) are shown 5–10 min pretetanus and 20–30 min posttetanus for each condition; calibration bar 2 mV; 5 ms. **A:** a 200-Hz tetanus induces a long-term potentiation (LTP) with an average increase in initial slope of 80.1 $\pm$ 10.2$\%$ over 1 h. This LTP was later determined to be mediated by both N-methyl-D-aspartate receptor/channels (NMDARs) and voltage-dependent calcium channels (VDCCs) and was thus named compoundLTP. **B:** a 200-Hz tetanus in the presence of 0.1 mg/kg MK-801, resulted in an LTP with an average increase in fEPSP of 35.7 $\pm$ 3.8$\%$ over 1 h. This LTP was mediated by VDCCs and was thus named vdccLTP. **C:** a 200-Hz tetanus in the presence of 10 mg/kg Verapamil resulted in an attenuated LTP; 37.2 $\pm$ 8.3$\%$ increase in initial slope of the fEPSP. This form of LTP was mediated by NMDARs and was thus named nmdaLTP. **D:** a 200-Hz tetanus in the presence of both 10 mg/kg Verapamil and 0.1 mg/kg MK-801 resulted in a total blockade of LTP. This blockade of LTP indicates that under these conditions LTP is mediated by NMDARs and VDCCs.
posttetanus period, of 80.1 ± 10.2% (mean ± SE, n = 8). This LTP was later determined by pharmacological manipulation to be mediated by both VDCCs and NMDARs and was named compoundLTP. The compoundLTP developed quickly and reached maximum magnitude at ~5 min posttetanus. The potentiated response then decayed slowly until stabilized at ~30–35 min posttetanus and remained stable for the 60-min recording period (Fig. 1A).

We examined the effects of the NMDAR antagonist MK-801 by administering 0.1 mg/kg ip 1 h prior to an identical tetanus. MK-801 had no effect on baseline responses as monitored for 1 h (data not shown). The initial slope of the posttetanus response increased by an average of 35.7 ± 3.8% and reflects the presence of vdccLTP (n = 5). The vdccLTP developed rapidly and increased gradually for the first 15–20 min from initial levels. Posttetanus responses then remained stable for the duration of the 60-min recording period (Fig. 1B).

Verapamil applied alone (10 mg/kg), 1 h before tetanus, resulted in an nmdaLTP with a posttetanus average increase in slope of 37.2 ± 8.3% (n = 5). This NMDAR-mediated potentiation can be characterized as rapidly developing and decaying slowly to become stable at 10–15 min posttetanus (Fig. 1C).

Injection of both MK-801 (0.1 mg/kg) and Verapamil (10 mg/kg) 1 h before tetanus completely eliminated LTP (n = 5), indicating the involvement of both VDCCs and NMDARs in the compoundLTP (Fig. 1D). Figure 2 compares the compoundLTP elicited in the absence of drugs, the isolated nmdaLTP and vdccLTP, and the complete block of LTP when both drugs are applied. As found previously (Cavus and Teyler 1996) the magnitude of compoundLTP (80.1 ± 10.2%) was approximately equal to the sum of vdccLTP (35.7 ± 3.8%) and nmdaLTP (37.2 ± 8.3%; Fig. 3).

In area CA1 of hippocampal slices, it has been shown that LTP under certain stimulation conditions is mediated by the activation of both NMDARs and VDCCs (Aniksztejn and Ben-Ari 1991; Cavus and Teyler 1996; Grover and Teyler 1990). Here we discuss the contribution of NMDARs and VDCCs to compoundLTP in the intact animal. Application of MK-801 reduced the magnitude of LTP by approximately half that of compoundLTP (Figs. 2 and 3). The lack of a full blockade of LTP by MK-801 indicates that NMDARs mediated part of the compoundLTP, but another mechanism may have contributed to the remaining potentiation. Application of only the VDCC antagonist Verapamil reduced the magnitude of the potentiation by approximately half (Fig. 2). Because Verapamil partially blocked compoundLTP we conclude that VDCCs contribute to compoundLTP. When both MK-801 and Verapamil are administered, complete block of compoundLTP results, providing further support for the role of NMDARs and VDCCs in compoundLTP (Fig. 1D). Because the magnitude of compoundLTP equals the sum of nmdaLTP and vdccLTP, our data do not offer strong support for an interaction between the 2 forms of LTP.

**DISCUSSION**

In area CA1 of hippocampal slices, it has been shown that LTP under certain stimulation conditions is mediated by the activation of both NMDARs and VDCCs (Aniksztejn and Ben-Ari 1991; Cavus and Teyler 1996; Grover and Teyler 1990). Here we discuss the contribution of NMDARs and VDCCs to compoundLTP in the intact animal. Application of MK-801 reduced the magnitude of LTP by approximately half that of compoundLTP (Figs. 2 and 3). The lack of a full blockade of LTP by MK-801 indicates that NMDARs mediated part of the compoundLTP, but another mechanism may have contributed to the remaining potentiation. Application of only the VDCC antagonist Verapamil reduced the magnitude of the potentiation by approximately half (Fig. 2). Because Verapamil partially blocked compoundLTP we conclude that VDCCs contribute to compoundLTP. When both MK-801 and Verapamil are administered, complete block of compoundLTP results, providing further support for the role of NMDARs and VDCCs in compoundLTP (Fig. 1D). Because the magnitude of compoundLTP equals the sum of nmdaLTP and vdccLTP, our data do not offer strong support for an interaction between the 2 forms of LTP (Grover and Teyler 1992). We conclude that, in the intact preparation, compoundLTP can be elicited by an afferent tetanus and each form of LTP can be pharmacologically separated.

LTP experiments have been conducted in area CA1 in vivo in the past and have not described the VDCC component of LTP, concluding that the LTP was solely mediated by NMDARs. One possible explanation for this may be the different tetanus parameters used to induce LTP by various investigators. During tetanus the cell must remain in a sustained depolarized state to induce vdccLTP (Grover and Teyler 1990). A 200-Hz tetanus results in sustained depolarization and induces vdccLTP, whereas a 25-Hz tetanus does not result in sustained depolarization and will induce nmdaLTP but not vdccLTP (Grover and Teyler 1990). Studies that use a 100- or 200-Hz tetanus and find a complete block of LTP by antago-
nists to the NMDAR may be stimulating with a voltage that is not great enough to elicit a sustained depolarization, which is needed for vdccLTP. During this study we noted that to elicit population spikes and presumably sustained depolarization, the tetanus voltage must be increased from low magnitude baseline stimulation to a slightly higher magnitude stimulation to elicit vdccLTP. This may be explained by the relatively large depolarization (positive values to −10 mV) that has to occur to allow dihydropyridine-sensitive VDCCs to open (Tsein et al. 1988). NMDARs in the absence of antagonists may act to help the cell depolarize enough to allow VDCCs to open. In the case where NMDARs are blocked, cell depolarization depends mostly on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) currents to depolarize the cell to a level sufficient to activate VDCCs.

Given the more demanding activation requirements of vdccLTP, is it reasonable to see vdccLTP outside the laboratory? Studies of hippocampal physiology suggest that these requirements may be met in nature. The axons of pyramidal neurons in area CA3 (Schaffer collaterals) produce a sharp wave (population excitatory postsynaptic potential) that is projected onto the dendrites of CA1 pyramidal cells and interneurons. Sharp waves observed in the Schaffer collaterals of area CA1 are short, aperiodic field potentials of a large magnitude (1–3 mV) (Buzsaki 1986; Buzsaki et al. 1983; Suzuki and Smith 1987). The consequence of this high-frequency, high-magnitude sharp wave is to drive neurons and interneurons into a 200-Hz phase-related discharge in the CA1 network (Buzsaki et al. 1992). Therefore the tetanus used here to induce vdccLTP may not be an uncommon event in the awake behaving animal.

The kinetics of vdccLTP recorded in this experiment approximated previous work in slices (Cavus and Teyler 1990; Grover and Teyler 1990) in that vdccLTP had a slow onset kinetic, reaching its maximum slope in ~5–15 min. In the current study, vdccLTP reached maximum slope in 10–15 min and remained constant for the duration of the experiments; however, the increase in slope from 1 min to 15 min posttetanus was minimal. Grover and Teyler (1990) noted that in vdccLTP they saw a large posttetanic potentiation (PTP) that quickly decayed in 2–3 min before the gradual increase in response occurred. Here, vdccLTP induction resulted in an immediate increase in slope that slowly increased over time. Although it is possible that these differences are due to the complexity and/or integrity of the two preparations, it is also possible that the use-dependant, open channel blocker antagonist we used (MK-801) may differ in its ability to attenuate LTP, as compared with the competitive receptor antagonist (APV) used in other studies. Another possibility is that the dose of MK-801 may have been insufficient to block the activation of the NMDA receptors completely. Although possible, this seems unlikely as the effect of both drugs together was to block LTP induction completely.

Much work has been done to elucidate the mechanism(s) of expression of LTP. Some of the proteins implicated in the phosphorylation cascade include the following: serine/threonine kinases [protein kinase C (PKC), protein kinase A (PKA)], calcium-calmodulin kinase II (CaMKII), and metabotropic glutamate receptors (mGluRs) and tyrosine kinases (Baudry et al. 1993). The broad spectrum serine threonine kinase inhibitor, H-7, blocks nmdaLTP but not vdccLTP (Grover and Teyler 1995). VdccLTP is blocked by Lavendustin A and/or Genestein, which are both tyrosine kinase inhibitors, but not by serine/threonine kinase inhibitors (Cavus and Teyler 1996). The dependence of nmdaLTP on serine/threonine kinases and vdccLTPs dependence on tyrosine kinases hints at the distinct transduction cascades that likely underlie the two forms of LTP. Entry of Ca$^{2+}$ through these two types of channels likely initiates two separate, although overlapping, phosphorylation cascades, that may differentially affect gene regulation (Ghosh and Greenberg 1995). This differential gene regulation may underlie important learning and/or memory mechanisms that have yet to be elucidated. Further work will help clarify the role(s) of these two forms of LTP in learning and memory.

The present findings provide electrophysiological evidence for the involvement of both VDCCs and NMDARs in tetanus-induced LTP in the intact animal. We conclude that like LTP in vitro, VDCCs and NMDARs underlie two separate forms of LTP in vivo.

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