Protein Synthesis Inhibition Blocks the Induction of Mossy Fiber Long-Term Potentiation *In Vivo*

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Protein synthesis inhibitors block the maintenance of NMDA receptor-dependent long-term potentiation (LTP) both *in vivo* and *in vitro*. Protein synthesis inhibitors block mossy fiber (MF) LTP maintenance *in vitro*, but little is known about the effect of protein synthesis inhibitors on either induction or maintenance in MF-LTP *in vivo*. Here we study the role of protein synthesis in the induction of long-term potentiation at the mossy fiber–CA3 hippocampal synapse *in vivo* in anesthetized rats. The protein synthesis inhibitor anisomycin was administered at different doses (0.04, 10, or 40 nmol) into area CA3 15 min before delivering high-frequency stimulation (two times at 100 Hz, 1 sec). Anisomycin blocked MF-LTP induction in a dose-dependent manner; both 40 and 10 nmol blocked MF-LTP induction, but a lower dose of 0.04 nmol was without effect. The inhibitory effect of

anisomycin on protein synthesis was determined by measuring the incorporation of [³⁵S]methionine into the newly synthesized proteins. Percentages of protein synthesis inhibition were determined by comparing [³⁵S] incorporation of anisomycin-treated samples with vehicle controls. Doses of 0.04, 10, or 40 nmol of anisomycin produced 21, 82, or 83% inhibition of [³⁵S]methionine incorporation, respectively. The effect of anisomycin was verified using a single dose of the protein synthesis inhibitor cycloheximide (40 nmol). Cycloheximide also blocked MF-LTP induction. These results suggest that protein synthesis plays an important role in the induction of mossy fiber long-term potentiation *in vivo*.

Key words: anisomycin; CA3; mossy fibers; hippocampus; long-term potentiation; cycloheximide and opioids

Long-term potentiation (LTP) refers to a long-lasting increase in amplitude of synaptic responses evoked in a monosynaptic pathway after high-frequency activation of specific afferent fibers (Bliss and Lomo, 1973). LTP is observed in the hippocampus, a brain structure associated with learning and memory (Scoville and Milner, 1957), suggesting that LTP is associated with learning and memory processes (Teyler and Discenna, 1984). The hippocampus displays at least two forms of LTP, one that is NMDA receptor-dependent (Harris et al., 1984) and one that NMDA receptor-independent and opioid receptor-dependent (Harris and Cotman, 1986; Derrick et al., 1991). NMDA receptor-dependent LTP is comprised of two processes, induction and maintenance. The maintenance process has two phases, an early phase (1–3 hr), which is protein synthesis-independent, and a late phase (>3 hr), which is protein synthesis-dependent (Krug et al., 1984; Frey et al., 1988).

Most of the studies investigating the mechanisms of LTP focused on the synapses that display NMDA receptor-dependent LTP. Both *in vivo* and *in vitro* studies demonstrate that the mossy fiber (MF)–CA3 synapse displays a form of LTP (MF-LTP) that is NMDA receptor-independent (Harris and Cotman, 1986; Johnston, 1992) and requires opioid receptor activation (Derrick et al., 1991) (but see Salin et al., 1995). Regarding the mechanisms involved in MF-LTP induction, studies suggest that Ca²⁺ entry is necessary for MF-LTP induction (Williams and Johnston, 1989; Zalutsky and Nicoll, 1990). It is reported that the increase in Ca²⁺ results in the activation of a second messenger cascade that involves calcium/calmodulin-dependent adenylyl cyclase activation (Huang et al., 1994; Weisskopf et al., 1994), a rise in cAMP, and the activation of cAMP-dependent protein kinase (Weisskopf et al., 1994).

Recent findings *in vitro* report that MF-LTP similarly expresses

an early-protein-independent and a late-protein-dependent phase (Huang et al., 1994). The time course of MF-LTP measured *in vivo* is also different from that measured *in vitro*. The asymptote of MF-LTP potentiation *in vitro* is observed immediately after the delivery of the trains (Jaffe and Johnston, 1990; Huang et al., 1994; Weisskopf et al., 1994; Urban and Barrionuevo, 1996). In contrast, MF-LTP *in vivo* is characterized by an initial potentiation, which develops slowly over time and only reaches a maximum augmentation in EPSP slope in ~1 hr after high-frequency stimulation (Derrick et al., 1991). This suggested to us that the processes underlying the induction of MF-LTP measured *in vivo* may be different from that measured *in vitro*.

In the present study, we tested the hypothesis that MF-LTP induction is fundamentally different from that observed in both NMDA receptor-dependent systems and MF-LTP induced *in vitro* by characterizing the effect of the protein synthesis inhibitors anisomycin and cycloheximide on the induction of MF-LTP in *in vivo* anesthetized rats. We report that protein synthesis inhibition blocks the induction of MF-LTP in a dose-dependent manner. These findings suggest that, *in vivo*, protein synthesis is necessary for MF-LTP induction.

MATERIALS AND METHODS

Animals. Adult male Sprague Dawley rats (Simonsen, Gilroy, CA and Harlan Sprague Dawley, Indianapolis, IN), weighing 350–400 gm on arrival, were housed individually, with food and water available *ad libitum*. The animals were maintained on a 12 hr light/dark cycle. Animals were anesthetized with Nembutal (65 mg/kg, i.p.) and given supplemental pentobarbital injections (6.5 mg/kg) at 1 hr intervals to maintain a surgical level of anesthesia. Body temperature was maintained at 37°C with a heating pad.

Extracellular mossy fiber CA3 evoked responses. A 33 ga stainless steel cannula–recording electrode was placed above the CA3 pyramidal layer of the dorsal hippocampus [anteroposterior (AP), –2.9 mm; mediolateral (ML), 2.2 mm (Paxinos and Watson, 1986)] using a stereotaxic instrument (Kopf, Tujunga, CA). The combination of cannula–recording electrode was constructed by insulating (Epoxytite, Irvine, CA) the outside of the cannula, except at the tip and 3 cm from top. In the top noninsulated area, a stainless steel wire was wrapped around the cannula and connected to the amplifier via an amphenol connector. Plastic tubing was attached to the top aperture, and it was used to deliver the drugs. This cannula–recording electrode allowed us to deliver drugs exactly in the same area in which the evoked responses were collected. Responses were evoked via direct stimulation of the mossy fibers using a stainless steel bipolar stimulating

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electrode (0.005 inch diameter), at coordinates corresponding to the orientation of mossy fiber projections (AP, -3.5 mm; ML, 2.0 mm). Constant current stimulation (10 – 50 μ A monophasic pulses, 0.2 msec duration) was provided by a Grass S48 stimulator, delivered to the stimulating electrode through a Grass Stimulus Isolation Unit (PSIU6).

Dorsoventral coordinates for the stimulating and recording electrodes were determined as described previously (Derrick et al., 1991). An electrode was placed initially in the granule cell layer of the dentate gyrus with the aid of stereotaxic coordinates and audio monitoring of CA1 pyramidal cell and dentate granule cell injury-induced unit discharges. A second electrode then was lowered into the CA3 region (3.1 – 3.3 mm below the brain surface). Stimulation was delivered at a rate of 0.35 Hz until antidromic spikes resulting from mossy fiber stimulation (2 – 3 msec to peak) were observed in the dentate. Orthodromic responses then were evoked by delivering stimulation through the dentate electrode and recording from the CA3 electrode. The stimulating electrode was adjusted until a characteristic mossy fiber field EPSP was observed consisting of a small (~ 0.5 mV) negative potential that is easily elicited with low (10 – 50 μ A) current intensities and that displays an onset of 3 – 4 msec and a peak at ~ 8 – 10 msec. Synchronous mossy fiber population spikes superimposed on field EPSPs were not observed, either at relatively high stimulation intensities (>70 μ A) or after high-frequency stimulation.

The evoked responses were amplified on a Grass P5 series alternating current preamplifier, filtered at 0.1 Hz– 3 kHz, digitized (10 points/msec) using a microcomputer, and then stored for off-line analysis using DataWave software (DataWave Technologies, Longmont, CO). The current intensity that elicited a 50% maximal response in each animal was determined and used for all subsequent stimulation, including high-frequency stimulation to induce LTP. To measure treatment effects, responses were evoked once every 20 sec throughout the entire experiment, and slope measures were calculated.

Drug treatment. After a 20 min baseline period, anisomycin (0.04 , 10 , or 40 nmol; 1 μ l total volume) (Sigma, St. Louis, MO) or cycloheximide (40 nmol; 1 μ l total volume) (Sigma), prepared in lactated Ringer's solution, were delivered into the stratum lucidum of hippocampal area CA3 via pressure ejection. One group of animals received anisomycin in the stratum lucidum of the contralateral CA3 to ensure that any effect observed was not attributable to anisomycin diffusion outside of the tetanized pathway. To ensure that the recordings were from mossy fibers-evoked responses, a group of animals received the NMDA-receptor blocker ($\pm$)-(3-(2-carboxypiperazin-4-yl) (CPP) (10 mg/kg, i.p.). This dose of CPP blocks NMDA receptor-dependent LTP *in vivo* (Hernandez et al., 1994). For both protein synthesis inhibitors used, the solution was delivered over a 5 min period, using a cannula-recording electrode. This drug infusion period was followed by a 15 min postdrug period and then by the delivery of high-frequency stimulation. LTP was induced by delivering high-frequency stimulation of two 100 Hz trains, with an intertrain interval of 20 sec (200 total pulses). To measure the development of LTP, evoked responses were collected for an additional 1 hr.

Verification of electrode placement. Electrode placement was verified using both electrophysiological and histological criteria. The electrophysiological criteria involved the audio localization of CA1, CA3, and dentate gyrus cell fields, the observation of an evoked antidromic response in the dentate gyrus (observed when stimulating CA3 and recording in the dentate gyrus), and the presence of mossy fiber-evoked responses preceded by a presynaptic volley (observed when stimulating in the dentate gyrus and recording in area CA3). Ten percent of the animals were subjected to histological analysis. The brains were extracted, frozen, and cut coronally at a thickness of 40 μ m using a microtome. Once the electrode tracks were visible, every fifth section was saved and mounted on slides. The tracks made by the stimulating and recording electrodes were reconstructed by displaying the section with a projection microscope onto photocopies of the rat brain stereotaxic atlas. For these animals, the electrodes were correctly placed 100% of the time.

Measurement of protein synthesis inhibition. Anisomycin was dissolved in an aqueous solution containing [35 S]methionine (10 μ Ci/ μ l; NEN, Boston, MA) and 10 mM β -mercaptoethanol, a stabilizer. Drug administration and electrophysiological procedures were the same as described above. The final dose of anisomycin administered was 0.04 , 10 , or 40 nmol. One hour after the delivery of high-frequency stimulation, the animal was decapitated, and the entire hippocampus, ipsilateral to the stimulation–recording site, was quickly dissected on ice-cold PBS, pH 7.0 . Protein synthesis inhibition was measured using similar methods as described previously (Otani et al., 1989). The tissue sample was homogenized by sonication in 5 vol of cold protease-free lysis buffer (5% SDS, 312 mM Tris-Cl, 312 mM imidazole, 200 mM dithiothreitol, and 50% glycerol) and further diluted in 5 vol of protease-free water, followed by boiling for 2 min. Cellular debris was removed by centrifugation (7000 rpm for 20 min at room temperature), and the supernatant was collected. Total radioactivity in the supernatant was determined and compared with radiolabel incorporated into the trichloroacetic acid precipitable cellular protein fraction (Bonifacio, 1993). The percent ratio of these two samples (TCA and non-TCA treated) was calculated for each group. The percent ratio calculated for each sample was expressed as the percentage value of the Ringer's solution-treated animals (Ringer's–anisomycin/Ringer's $\times 100$).

Whole-cell recording. Hippocampal slices (300 μ m) were harvested from 15 – 30 -day-old Sprague Dawley rats and maintained *in vitro* as described

previously (Chitwood and Jaffe, 1998). Slices were maintained at room temperature in oxygenated (95% O_2 – 5% CO_2) artificial CSF (aCSF) containing (in mM): 124 choline chloride, 2.5 KCl, 26 NaHCO $_3$, 3 MgCl $_2$, 3 CaCl $_2$, 1.25 NaHPO $_4$, and 10 dextrose. Slices were transferred as needed to a submersion-type recording chamber perfused with oxygenated aCSF (~ 1 ml/min), also at room temperature.

The recording chamber was mounted on an upright microscope (Axioskop; Zeiss, Oberkochen, Germany) to visualize CA3 pyramidal neurons using infrared video–differential interference contrast microscopy (Stuart et al., 1993). Whole-cell patch-clamp recordings were made using pipettes containing (in mM): 150 K-gluconate, 20 KCl, 0.1 – 1 EGTA, 2 MgCl $_2$, 2 Na $_2$ ATP, and 10 HEPES, pH 7.3 . Electrodes were backfilled with the same solution containing 100 μ M the calcium-sensitive dye fura-2. Electrical recordings were measured using an Axoclamp 2b (Axon Instruments, Foster City, CA) in bridge current-clamp mode and digitized using an Instrutech ITC-16 board (Instrutech, Great Neck, NY) connected to a Power Macintosh computer (Apple Computer, Cupertino, CA) running AxoData (Axon Instruments) acquisition software. Analysis of electrical data were performed using custom software written with Igor Pro (Wave-metrics, Lake Oswego, OR).

Fura-2 was excited by a xenon lamp at 380 nm, and emission was detected at wavelengths greater than 512 nm using a cooled CCD camera (PXL-37; Photometrics, Tucson, AZ) in frame transfer mode. Software for this camera was provided by Dr. Joseph Callaway (University of Tennessee, Memphis, TN). Changes in intracellular calcium were determined at a single excitation wavelength (380 nm) by normalizing changes in fluorescence (ΔF) to resting fluorescence levels (F) and reported as percent $\Delta F/F$. Autofluorescence was determined from a nondendritic region lacking any changes in fluorescence signal or from fluorescence sequences taken 200 – 300 μ m away from the filled cell with the same laminar register within CA3. Bleaching was corrected by subtracting control images without electrical or synaptic stimulation. Calcium imaging, electrical stimulation, and data acquisition were coordinated using the Master-8 stimulus generator (A.M.P.I., Jerusalem, Israel).

Data analysis. The effect of the protein synthesis inhibitor anisomycin on the induction of MF-LTP was measured by comparing the percent change in the EPSP slope in the last 5 min of the baseline period with the last 5 min of the 1 hr collection period. This percent change was calculated using a trimmed mean. Inherent in *in vivo* recordings are physiological artifacts (heartbeat, synchronous firing of pyramidal cells) that sporadically can alter evoked responses slopes. Trimming of means is an accepted technique (Wainer, 1982) for reducing sample variability and minimizing the contribution of these sources of spurious signals. The calculation of the trimmed mean is conducted by eliminating the highest and the lowest numbers from the distribution, including the last 5 min of the baseline and the last 5 min of the 1 or 2 hr (CPP-treated group) collection period. The significance of the percent change values in the EPSP slope and the percent changes of [35 S]methionine incorporation was determined using a one-way ANOVA. Data from the groups that received cycloheximide, CPP alone, nonpotentiated control with anisomycin, or anisomycin in the contralateral CA3 side were analyzed using a dependent Student's *t* test to determine whether post-treatment and post-tetanization responses were different from those recorded during the baseline period. An independent Student's *t* test was used to compare post-tetanization responses between the group that received anisomycin in the contralateral CA3 and the Ringer's solution-treated control. A one-way repeated measure ANOVA was used to analyze the anisomycin-treated but not potentiated group. Measurements were obtained at 20 , 40 , and 80 min periods.

RESULTS

In these experiments, we investigated whether protein synthesis is required for the induction of MF-LTP *in vivo*. The protein synthesis inhibitor anisomycin was delivered into hippocampal area CA3 before administration of high-frequency stimulation. We found that anisomycin blocked the induction of mossy fiber long-term potentiation in a dose-dependent manner. There was no significant difference found between Ringer's- and anisomycin- (0.04 nmol) treated animals with the lowest dose ($F_{(1,8)} = 0.924$; $p > 0.05$). Both groups displayed MF-LTP that lasted for the duration of the experiment (Fig. 1A,B).

Both 10 nmol ($F_{(1,8)} = 6.709$; $p < 0.03$) and 40 nmol ($F_{(1,10)} = 10.00$; $p < 0.01$) of anisomycin effectively blocked the induction of MF-LTP when compared with Ringer's control (Fig. 1C,D). There were no significant differences between the animals that received either 10 or 40 nmol of anisomycin ($F_{(1,10)} = 0.042$; $p > 0.05$).

The inhibitory effect of anisomycin on protein synthesis was determined by measuring the incorporation of [35 S]methionine into newly synthesized proteins. Anisomycin at doses of 0.04 , 10 , and 40 nmol resulted in 21 , 82 , and 83% protein synthesis inhibition, respectively. Both 10 nmol ($F_{(1,4)} = 9.284$; $p < 0.04$) and 40 nmol ($F_{(1,4)} = 8.856$; $p < 0.04$) of anisomycin produced significant

Figure 1. Inhibition of protein synthesis by anisomycin blocks the induction of MF-LTP. *A*, Induction of MF-LTP after the injection of Ringer's solution ($n = 5$). *B*, A low dose of anisomycin (0.04 nmol) does not block the induction of MF-LTP ($n = 7$), whereas higher doses of 10 ($n = 5$) or 40 ($n = 7$) nmol block its induction (*C*, *D*). Representative traces for each panel were taken 1 min before and 60 min after high-frequency stimulation. Calibration: 0.5 mV, 5 msec.

Figure 2. The effect of anisomycin is localized ipsilateral to the injected side. *A*, Administration of anisomycin into the CA3 region of the contralateral hippocampus had no effect on MF-LTP ($n = 4$). *B*, MF-LTP is induced in animals that received the NMDA receptor antagonist CPP (10 mg/kg, i.p.). *C*, Injection of the protein synthesis inhibitor cycloheximide (40 nmol) also blocked the induction of MF-LTP ($n = 5$). *D*, Anisomycin does not affect low-frequency evoked responses. Representative traces for each panel were taken 1 min before and 60 or 120 (CPP group) min after high-frequency stimulation. Calibration: 0.5 mV, 5 msec.

decreases in the level of protein synthesis compared with subjects treated with [35 S]methionine. The level of protein synthesis inhibition produced by the dose of 0.04 nmol was not significantly different from control ($F_{(1,4)} = 0.591$; $p > 0.05$).

Anisomycin (40 nmol) delivered into the contralateral CA3 region did not block the induction of MF-LTP ipsilateral to the stimulation side ($t = -2.784$; $df = 4$; $p < 0.05$) (Fig. 2*A*). Furthermore, there was no significant difference found between the group that received anisomycin in the contralateral CA3 and the Ringer's

solution-treated control ($t = -2.303$; $df = 8$; $p > 0.05$). MF-LTP was observed in animals that received the NMDA receptor antagonist CPP ($t = -6.871$; $df = 3$; $p < 0.05$) (Fig. 2*B*), and the potentiation lasted for the entire length of the experiment (2 hr). The protein synthesis inhibitor cycloheximide at a concentration of 40 nmol also blocked the induction of MF-LTP ($t = 0.370$; $df = 3$; $p > 0.05$) (Fig. 2*C*). A one-way ANOVA found no significant changes in the anisomycin-treated nonpotentiated group ($F_{(2,6)} = 4.130$; $p > 0.05$) (Fig. 2*D*).

Figure 3. Anisomycin does not affect voltage-gated calcium entry. *A*, Micrograph of a CA3 pyramidal neuron. The patch electrode filled with fura-2 is visible at the top. The three squares represent regions of interest for measuring changes in fluorescence at the soma, ~100 and 200 μm from the soma. Scale bar, 100 μm. *B*, Time course of somatic $\Delta F/F$ before and during the application of 100 μM anisomycin. *C*, Calcium transients at the soma, 100 and 200 μm from the soma in response to 10 action potentials before (*C*₁) and after (*C*₂) exposure to anisomycin.

To determine whether anisomycin has nonspecific effects on voltage-gated calcium entry and, in turn, on the potential induction of MF-LTP (Kapur et al., 1998), we next performed experiments using the *in vitro* hippocampal slice preparation. With the use of standard fluorescence imaging methods, spike-mediated calcium entry into CA3 pyramidal neurons was monitored before and after exposure to 100 μM bath-applied anisomycin ($n = 3$). As illustrated in Figure 3, voltage-gated calcium influx into both the soma and dendrites, in response to 10 action potentials, was not affected by exposure to anisomycin.

DISCUSSION

The present study reveals that anisomycin, when administered 15 min before the delivery of high-frequency stimulation, blocks the induction of MF-LTP in a dose-dependent manner. The inhibitory effect of anisomycin on protein synthesis inhibition was confirmed by measuring the incorporation of [³⁵S]methionine into newly synthesized proteins. The two effective doses of anisomycin, 10 and 40 nmol, resulted in 82 and 83% of protein synthesis inhibition, respectively. The percentages of inhibition effective in blocking MF-LTP induction are comparable with those observed in studies in which protein synthesis inhibition blocked maintenance (Otani et al., 1989) of NMDA receptor-dependent LTP in the dentate gyrus.

It is possible that synaptic responses might be elicited via commissural/association (C/A) fibers caused by the direct activation of CA3 pyramidal neurons by axon collaterals extending into the hilar region (Li et al., 1994). Because LTP at C/A synapses is NMDA receptor-dependent, one group received the NMDA receptor antagonist CPP (10 mg/kg). Here CPP did not affect baseline responses or the induction of LTP, thus demonstrating that the responses were primarily attributable to the activation of the mossy fibers.

Another explanation to the present results is that anisomycin spread to other areas and that the effect observed was not attributable to the localized administration into the mossy fiber–CA3 synapses but to the effect of protein synthesis inhibition in areas sending projections to the CA3 region, such as the commissural CA3 projections. To address this possibility, a group of animals received anisomycin in the contralateral CA3 region. In this group, anisomycin did not block MF-LTP. This finding indicates that the

effect of anisomycin on protein synthesis inhibition was localized to the ipsilateral hippocampus in which it was injected.

Alternatively, the effect of anisomycin on the induction of MF-LTP could be attributable to a nonselective action of anisomycin on the physiology of CA3 pyramidal neurons. However, the finding that the administration of another protein synthesis inhibitor, cycloheximide, also blocked the induction of MF-LTP suggests that the effect observed is attributable to protein synthesis inhibition. That is, the two drugs have the same primary action, protein synthesis inhibition, but a different spectrum of side effects. That they have the same effect on MF-LTP increases the confidence that the effect observed is attributable to their common primary action.

Additionally, we investigated whether anisomycin nonspecifically affected calcium entry into CA3 pyramidal neurons. For instance, if anisomycin affects calcium entry into the cell, this could also block MF-LTP, because calcium is necessary for LTP induction at this synapse (Williams and Johnston, 1989; Urban and Barriounevo, 1996; Kapur et al., 1998). Bath-applied anisomycin did not change calcium flux in pyramidal cells of hippocampal area CA3. Thus, the blockade of the induction of MF-LTP was not attributable to an effect on calcium dynamics.

Because these studies were conducted in the anesthetized animal, it is possible that Nembutal affected protein synthesis. Nembutal-induced protein synthesis inhibition could add to the inhibition observed in the anisomycin-treated animals and thus account for the blockade of MF-LTP. To our knowledge, Nembutal does not affect protein synthesis. All of the measurements of anisomycin-treated animals are compared with the Ringer's group that was also treated with Nembutal and showed LTP.

The findings in the present study are in contrast to those of Huang et al. (1994) who reported that anisomycin blocked the maintenance but not the induction of MF-LTP. The findings from this group may differ from the present results because of significant differences between mossy fiber-LTP induced either *in vivo* or *in vitro*. For instance, Huang et al. showed a rapid onset and decremental LTP when one 100 Hz train was applied and a rapid onset and nondecremental LTP when three 100 Hz trains were used. In contrast, in our *in vivo* experiments, nondecremental LTP was triggered by two 100 Hz trains and augmentation of the response had a very slow onset and took 1 hr to reach its maximum. Thus, the differences in time course might reflect dependence on protein synthesis that is observed during the induction of *in vivo* MF-LTP and not be required for the induction of *in vitro* MF-LTP.

The idea that local protein synthesis is involved in mediating synaptic plasticity in the MF-CA3 synapse is supported by the findings that mRNA for proteins such as CaM kinase II are present in the dendrites of this synaptic region (Chicurel et al., 1993). CaM kinase II is implicated in the induction of NMDA receptor-dependent LTP (Finn and Browning, 1980; Mody et al., 1984), and α CaM kinase II is implicated in learning and memory (Silva et al., 1992). It is possible that translation of such resident mRNAs may be activated with the stimulation parameters used in our study to induce MF-LTP and that new protein products are required for the development of LTP, which takes ~1 hr *in vivo*.

Because new protein synthesis is necessary for the formation of long-term memory (Bennett et al., 1977) and because LTP, a form of synaptic plasticity, may mediate the formation of long-term memory in the brain (Teyler and Discenna, 1984), it is important to understand the role of new protein synthesis in LTP. More importantly, similarities in the role of new protein synthesis in long-term memory and in the induction of LTP would provide convergent evidence supporting a role for LTP in learning and memory processes. In the behavioral literature, a critical level of protein synthesis is required for the formation of long-term memory (Bennett et al., 1977; Quinton and Kramarcy, 1977). An amnesic effect was observed after the administration of either anisomycin or cycloheximide, when the inhibition protein synthesis was over 80%. Interestingly, a critical level of 80% protein synthesis inhibition is necessary to block the induction of NMDA receptor-dependent LTP (Deadwyler et al., 1987). In agreement with these observa-

tions, it is shown here that both 10 and 40 nmol doses of anisomycin, which blocked induction of MF-LTP, produced over 80% inhibition of protein synthesis.

In summary, we found that the induction of MF-LTP *in vivo* requires protein synthesis, suggesting that the mechanisms underlying synaptic plasticity under these conditions are different from those observed during the induction of MF-LTP *in vitro*.

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