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Opioid Receptor Activation Is One Factor Underlying the Frequency Dependence of Mossy Fiber LTP Induction

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The contribution of high-frequency synaptic activity to the induction of long-term potentiation (LTP) in the opioid peptide–containing mossy fiber projection was investigated *in vivo* in anesthetized rats. Because high-frequency mossy fiber activity is essential for both the release of opioid peptides and the induction of mossy fiber LTP, we investigated whether the activation of opioid receptors underlies the requirement of sustained high-frequency mossy fiber activity for LTP induction. Mossy fiber responses were found to have a distinct threshold for the number of 100 Hz pulses necessary to induce LTP, with bursts of 25–30 pulses being the minimum for LTP induction. Application of 1 nmol of the μ-opioid receptor agonist DAMGO to the CA3 region potentiated mossy fiber responses, but, unlike for mossy fiber LTP, this potentiation could be reversed by μ-opioid receptor antagonist CTOP. Stimulation of the mossy fibers with either a single burst of 15 pulses at 100 Hz or application of 100 pmol of DAMGO was ineffective in potentiating mossy fiber responses. However, delivery of a 15 pulse burst 10 min following DAMGO application was effective in potentiating mossy fiber responses. This potentiation was not reversed by CTOP and it occluded stimulation-induced LTP, suggesting that brief bursts delivered in the presence of DAMGO had induced mossy fiber LTP. The release of opioid peptides and the resulting activation of μ-opioid receptors is suggested as one factor that underlies the requirement of sustained high-frequency stimulation for the induction of mossy fiber LTP. Because μ-opioid receptor activation alone is not sufficient to induce mossy fiber LTP, brief high-frequency stimulation apparently provides additional factors that also are necessary for the induction of mossy fiber LTP.

[Key words: CA3, CTOP, DAMGO, dentate gyrus, hippocampus, long-term potentiation, μ-opioid receptors]

Long-term potentiation (LTP) is an enduring change in synaptic efficacy that results from brief high-frequency afferent volleys (Bliss and Lomo, 1973; Bliss and Lynch, 1988). The specificity and associativity of LTP make it an ideal candidate for a cellular process that may underlie the storage of some types of information in the vertebrate brain (Teyler and DiScenna, 1987). At least two forms of LTP are expressed within the hippocampal formation. One form is sensitive to antagonists of the NMDA receptor and is found at many hippocampal synapses, including the Schaffer collateral projection to area CA1 (Collingridge et al., 1983) and medial perforant path afferents to the dentate gyrus (Errington et al., 1987) and area CA3 (Derrick et al., 1993). A second form of LTP found in the hippocampal formation is insensitive to NMDA receptor antagonists but is sensitive to opioid receptor antagonists, and is found exclusively in opioid peptide–containing projections such as the mossy fiber–CA3 projection (Martin, 1983; Harris and Cotman, 1986; Derrick and Martinez, 1989; Derrick et al., 1991), and the lateral perforant path projection to both the dentate gyrus and area CA3 of the hippocampus (Bramham et al., 1988, 1991a,b; Briendl et al., 1991; for review, see Bramham, 1992).

The NMDA receptor–independent form of potentiation observed at mossy fiber synapses does not display associativity with coactive afferents (Chattarji et al., 1989) or LTP when single afferent volleys are paired with postsynaptic depolarization (Jaffe and Johnston, 1990; Zalutsky and Nicoll, 1990). These findings, together with the report suggesting that mossy fiber LTP is independent of both postsynaptic depolarization and postsynaptic calcium influx (Zalutsky and Nicoll, 1990; Katsuki et al., 1991), led to the suggestion that mossy fiber LTP may utilize a presynaptic induction mechanism that is independent of postsynaptic factors. However, other studies indicate that mossy fiber LTP depends on postsynaptic depolarization (Jaffe and Johnston, 1990) and postsynaptic calcium (Williams and Johnston, 1989). It is suggested that the inability of mossy fiber LTP to display single-pulse associativity, rather than indicating an independence from postsynaptic processes, reflects a requirement for high-frequency mossy fiber activity for the induction of mossy fiber LTP. In this view, some factor(s) associated with repetitive mossy fiber activity is essential for the induction of LTP at this synapse (Jaffe and Johnston, 1990).

The release of opioid peptides by the mossy fibers is a likely candidate for such a frequency-dependent factor. The mossy fibers contain and release pro-enkephalin and prodynorphin-derived opioid peptides (Gall et al., 1981; McGinty et al., 1984; Chavkin et al., 1985a), and the activation of μ-opioid receptors is essential for LTP induction at this synapse (Derrick et al., 1992). In addition, in many neural systems the synaptic release of peptides requires repetitive synaptic activity (Bicknell, 1988; Peng and Horn, 1991). This also appears to be the case with opioid peptides released by the mossy fibers, because high-frequency activation is necessary for the release of endogenous μ-opioid receptor ligands (Wagner et al., 1990). Thus, one factor underlying the requirement for sustained synaptic activity for the induction of mossy fiber LTP may be the frequency-depen-

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dent release of opioid peptides and the subsequent activation of μ-opioid receptors (Derrick et al., 1991, 1992). In the present study we assessed the frequency dependence of mossy fiber LTP, and the contribution of μ-opioid receptor activation to this frequency dependency.

Materials and Methods

All experimental procedures were approved in advance by the Animal Care and Use Committee at the University of California at Berkeley, and are in accordance with NIH guidelines.

General techniques. Adult male Sprague–Dawley rats (350–450 gm; Simonsen Labs, Gilroy, CA) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and maintained at 37°C with a heating pad. A surgical level of anesthesia was maintained with supplemental injections of pentobarbital (10 mg/kg/hr).

Extracellular mossy fiber responses were recorded using a single Teflon-coated nichrome wire placed above the CA3 pyramidal layer of the dorsal hippocampus (AP −3.2, ML 2.2; Paxinos and Watson, 1982). Mossy fiber–CA3 responses were evoked via direct stimulation of the mossy fibers using constant current stimulation (10–100 μA, 0.2 msec duration) provided by a Grass P350 stimulator and delivered through a Grass stimulus isolation unit to a single Teflon-coated nichrome wire (0.062 mm diameter; Medwire, Chicago, IL) exposed only at the tip. Electrodes were oriented using coordinates corresponding to the orientation of mossy fiber lamellae (Rawlins and Green, 1977; Fig. 1*A*). A monopolar electrode was first placed in the granule layer of the dentate gyrus (AP −3.5, ML 2.0), and a second electrode was lowered slowly into the CA3 region. Upon penetration of the mossy fiber bundle, antidromic responses (2–3 msec to peak) resulting from mossy fiber stimulation were observed in the dentate granule cell layer (Fig. 1*B*, top). Orthodromic responses were then evoked by delivering current through the dentate electrode and recording from the CA3 electrode. This elicited a characteristic mossy fiber field EPSP (excitatory postsynaptic potential) consisting of a small (approximately 0.5 mV) negative potential preceded by a presynaptic volley that corresponded with the latency of the antidromic spike (Fig. 1*B*, bottom). Mossy fiber responses were elicited with low (10–50 μA) current intensities, displayed an onset of 3–4 msec and with a peak at approximately 8–10 msec, and phased reversed sharply upon penetration of the CA3 pyramidal layer. Synchronous mossy fiber population spikes superimposed on field EPSPs are occasionally observed following high-frequency stimulation. Because these population spikes can alter measurements of the peak magnitude of negative-going mossy fiber field EPSPs recorded in the stratum lucidum, response magnitude was measured using the initial slope of the field EPSP slope measured at 2–3 msec following response onset for all studies assessing the effect of high-frequency stimulation on mossy fiber responses.

Mossy fiber responses were evoked a rate of 0.25 Hz using a current intensity that elicited a response that was 50% of the maximal mossy fiber response. Mossy fiber responses were amplified on a Grass P3 series A.C. preamplifier, filtered at 0.1 Hz to 1 kHz, digitized (10,000 Hz) using a microcomputer, and then stored for off-line analysis.

Burst–response curves. The dependence of mossy fiber LTP on high-frequency stimulation was assessed by varying the duration (150–2000 msec) of 100 Hz trains to deliver bursts ranging from 15 to 200 pulses. Following collection of baseline mossy fiber responses for at least 15 min, a single burst of 15–200 pulses was delivered. Trains were delivered at the 50% maximal current intensity that was used to evoke low-frequency responses. The magnitude of LTP relative to baseline response magnitudes was assessed at 26–30 min following the burst. A single burst of 200 pulses was delivered 30–60 min later to ascertain the viability of the preparation. The animal was eliminated from the study if a 20% or greater increase in slope was not observed at 1 hr after delivery of either burst. In separate experiments in which assessing the effects of μ-opioid receptor antagonists on LTP induction, baseline responses were collected for at least 20 min, after which a single burst of 100 Hz stimulation was delivered. The magnitude of mossy fiber LTP then was assessed at 25–30 min posttetanus. If the increase in mossy fiber amplitude was less than 20%, another 100 Hz burst of longer duration was delivered. This pattern was continued until at least a 20% potentiation of the field EPSP slope was observed. In both experiments, EEG (electroencephalogram) was monitored for 1 min following delivery of all high-frequency trains. Seizures and/or afterdischarges following high-frequency stimulation were never observed in these experiments.

Drugs. The selective μ receptor agonist [D-Ala^2,N-Me-Phe^4,Gly-ol^5]-enkephalin (DAMGO; Handa et al., 1981; James and Goldstein, 1984; 100 pmol and 10 nmol; RBI, Natick, MA) and the μ receptor–selective antagonist Cys^2, Tyr^3, Orn^5, Pen^7 amide (CTOP; Gulya et al., 1986; 1 and 3 nmol; Peninsula Labs, Belmont, CA) were dissolved in lactated Ringer's and delivered locally to area CA3 via pressure ejection (0.1 or 1.0 μl volumes, 0.2 μl/min, 0.5 and 5 min injection times) through a 28 gauge cannula adjacent to the recording electrode and flush with the electrode tip. In studies requiring application of more than one drug at different times, a guide cannula was placed adjacent to the recording electrode. A smaller gauge cannula, which extended 0.5 mm beyond the guide cannula and was flush with the electrode tip, was inserted just prior to application of each drug and removed following completion of drug delivery. Responses were collected for 10 min following completion of drug delivery to allow assessment the effect of the drug on low-frequency mossy fiber responses. In all experiments assessing the effects of local drug application on LTP induction, conditioning trains were delivered 10 min following completion of drug delivery.

Studies using the selective and competitive NMDA receptor antagonist (±)-CPP (RBI, Natick, MA) employed systemic administration (10 mg/kg). This dose has been found previously by ourselves (Hernandez et al., 1992) and others (Abraham and Mason, 1988) to block completely the induction of NMDA receptor–dependent LTP when administered at least 1 hr prior to delivery of conditioning trains.

Data analysis. The magnitude of responses evoked following application of receptor-selective agonists and antagonists was measured using a 20% trimmed mean (Wainer, 1982) of the responses occurring between 6 and 10 min following completion of drug application. Trimmed means minimize the contribution of spurious signals resulting from physiological artifacts such as heartbeat and synchronous pyramidal cell bursts. The magnitude of evoked responses following tetanization was measured using a 20% trimmed mean of the field EPSP slope occurring between 26 and 30 min posttetanus. Changes in response amplitudes as a result of drug application or delivery of conditioning trains were expressed as percentage change relative to the 20% trimmed mean of response amplitudes observed in the last 5 min of the baseline period. The significance of these changes was evaluated with a one-way analysis of variance (ANOVA; Keppel and Zedeck, 1991). The effect of prior treatments on the ability of a subsequent 200 pulse train to induce LTP was assessed using a two-way ANOVA that compared the magnitude of the response to a 200 pulse train (between-groups measure) in animals in which LTP (defined as a 20% or greater increase in response) was or was not observed previously. The interaction term for this analysis was used to determine whether the initial treatment attenuated further development of LTP.

Electrode placements were verified using stereotaxic coordinates and electrophysiological criteria, including audio localization of CA1, CA3, and dentate granule cell layers; the evocation of an antidromic response in the dentate gyrus; correspondence of the onset of the orthodromically elicited presynaptic volley with that of the antidromic spike; the times of onset and peak of mossy fiber responses; and phase reversal of mossy fiber responses in the CA3 pyramidal layer. In addition, electrode placement was verified histologically in 10% of the animals. These animals were killed with an overdose of pentobarbital and perfused with 10% formaldehyde, and 50 μm brain sections were stained with cresyl violet. Accurate electrode placements in the CA3 region were observed in 100% of these animals.

Results

In the studies assessing the effect of delivery of single bursts of 15–200 pulses, the occurrence of mossy fiber LTP was significantly dependent on size of the burst [$F(5,31) = 4.48$, $p < 0.01$], with bursts of 30 or more pulses being necessary for induction of LTP (Fig. 2). These results are summarized in Figure 3. There were no significant differences in the magnitude of LTP produced by 30, 50, or 200 pulses [$F(2,22) = 0.283$, $p > 0.05$], indicating delivery of bursts of 30 or more pulses resulted in maximal mossy fiber LTP. Further, once LTP was induced, subsequent delivery of a 200 pulse burst resulted with little further potentiation [mean percentage change of mossy fiber

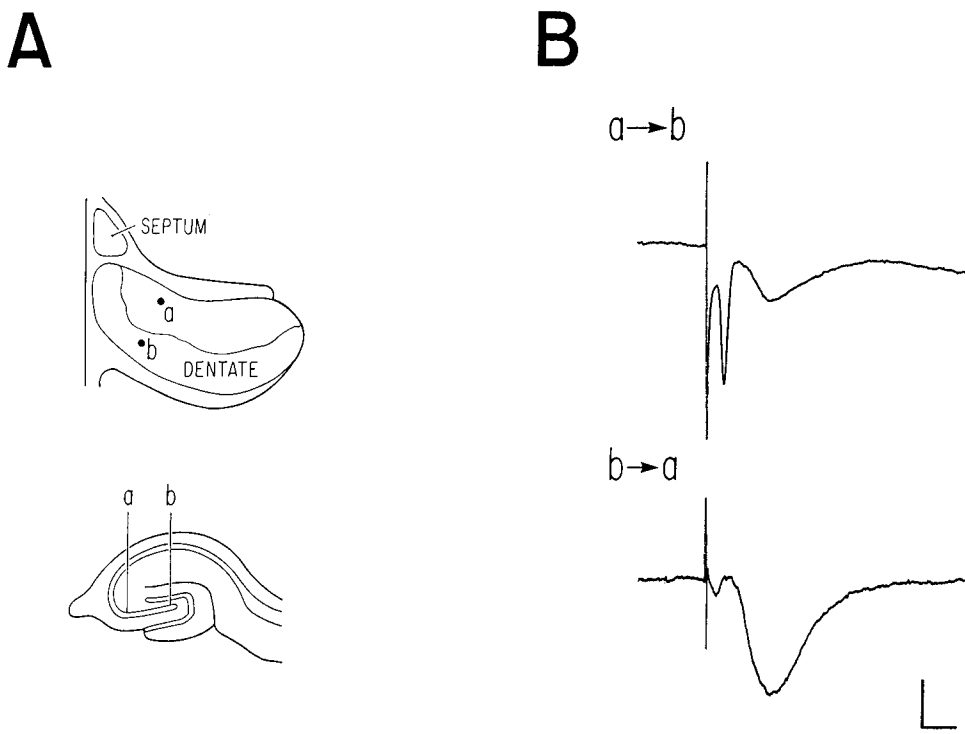

Figure 1. Schematic diagram of electrode placement for evoking mossy fiber responses *in vivo*. *A*, Electrode sites as viewed from horizontal (*top*) and sagittal (*bottom*) planes. Electrodes are placed in both the CA3 region (*a*) and the dentate gyrus (*b*). *B*, Representative responses evoked by antidromic activation of granule cells (stimulate site *a*, record in site *b*; *top trace*) and orthodromically evoked mossy fiber responses as recorded in the stratum lucidum (stimulate site *b*, record from site *a*; *bottom trace*). Calibration: *top*, 5 msec, 2.5 mV; *bottom*, 5 msec, 0.25 mV.

responses following bursts of 200 pulses = 67.4 ± 9%; additional potentiation of mossy fiber responses produced by a subsequent bursts of 200 pulses = 16.3 ± 16%, $F(1,16) = 27.4$, $p < 0.0001$].

Mossy fiber LTP induced by brief trains displayed characteristics that previously were reported with mossy fiber LTP induced by sustained trains. As we reported (Derrick et al., 1992), local application of the selective μ-opioid receptor antagonist CTOP (3 nmol) blocked mossy fiber LTP induction [mean percentage increase in mossy fiber field EPSP after delivery of 30 pulses in the presence of CTOP = −8 ± 10%, $F(1,5) = 8.42$, $p < 0.05$, as compared to LTP induced with 30 pulses in the absence of CTOP; Fig. 3]. Systemic administration of the NMDA receptor antagonist (±)-CPP 90 min prior to delivery of conditioning trains, which blocks LTP induction at synapses dependent on NMDA receptor activation (Abraham and Mason, 1988; Hernandez et al., 1992), had no effect on mossy fiber LTP induction [mean percentage increase in mossy fiber EPSP slopes following a 200 pulse train = 68 ± 19%, $F(1,10) = 0.36$, $p > 0.05$, as compared to LTP induced by 200 pulses in the absence of (±)-CPP; Fig. 3].

Local application of the μ-opioid receptor–selective agonist DAMGO (10 nmol) to the CA3 region increased the peak magnitude of mossy fiber field EPSPs evoked with low-frequency stimulation. This increase reflected primarily the appearance of synchronous negative-going population spikes superimposed on the field EPSPs. Consistent increases in the field EPSP slope were not observed. The excitatory effect of DAMGO was reversed by a 3 nmol quantity of the μ-opioid receptor–selective antagonist CTOP applied 20 min after DAMGO application ($n = 3$; Fig. 4).

Application of a smaller (100 pmol) quantity of DAMGO following collection of baseline responses failed to produce significant changes in mossy fiber response magnitude when measured at 6–10 min following completion of drug delivery. A 150 msec 100 Hz train (15 pulses), which by itself is ineffective in

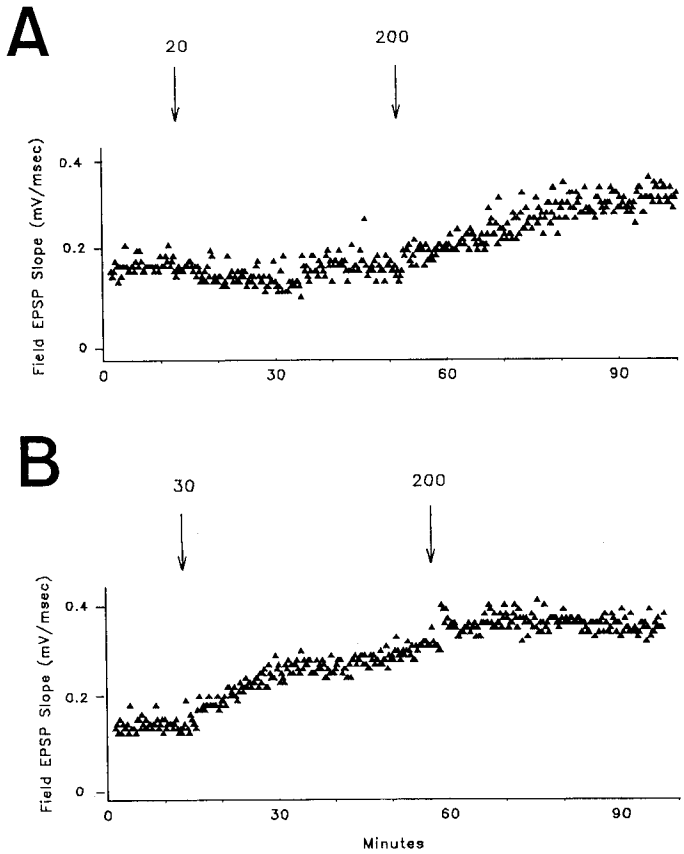

Figure 2. Representative plots of mossy fiber field EPSP slope magnitude following bursts delivered at 100 Hz. *A*, Potentiation of mossy fiber field EPSP slopes was evident after a single burst of 200, but not 20, pulses. *B*, A single burst of 30 pulses delivered at 100 Hz produced a potentiation of mossy fiber responses; a subsequent burst of 200 pulses at this frequency delivered 1 hr later produced little additional potentiation.

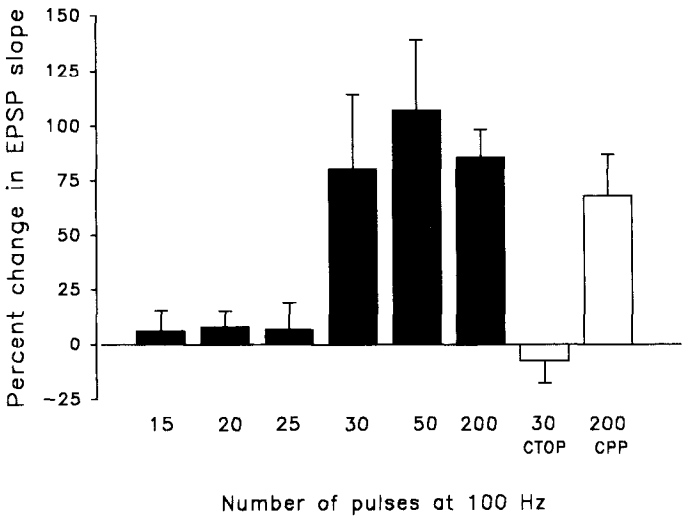

Figure 3. Summary of the change in mossy fiber field EPSP slope as a function of the number of pulses delivered as a single 100 Hz burst. Each bar represents the mean percentage change (±SEM) in mossy fiber field EPSP slope magnitude measured 26–30 min following high-frequency stimulation [*15*: 15 pulses, $n = 7$; *20*: 20 pulses, $n = 3$; *25*: 25 pulses, $n = 3$; *30*: 30 pulses, $n = 4$; *50*: 50 pulses, $n = 3$; *200*: 200 pulses, $n = 10$; *CTOP*: 30 pulses delivered 10 min following delivery of a 3 nmol quantity of CTOP, $n = 4$; *CPP*, 200 pulses delivered 1 hr following intraperitoneal administration of (±)-CPP (10 mg/kg), $n = 3$].

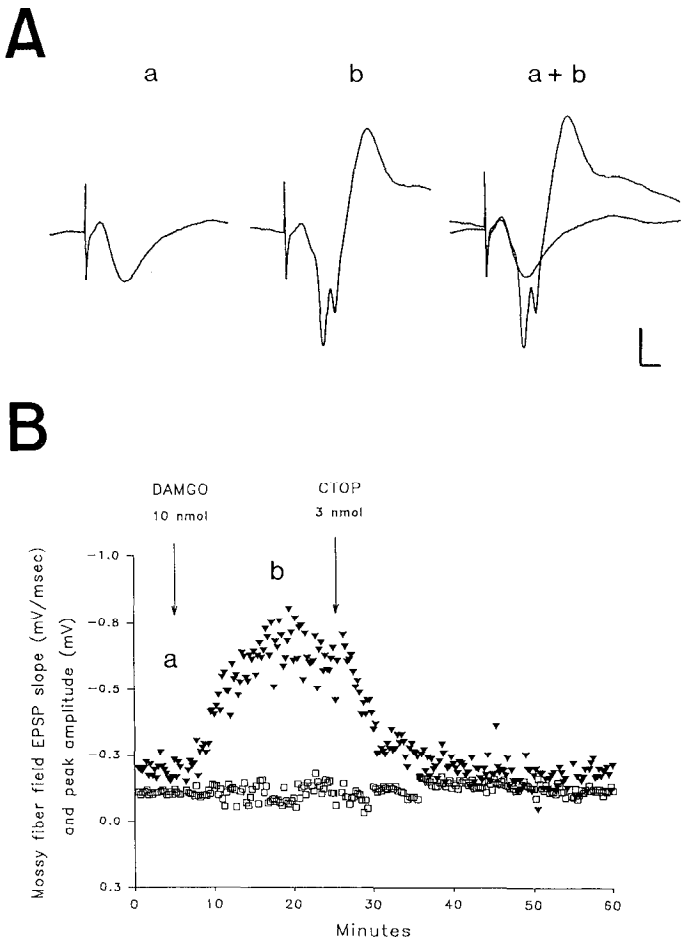

Figure 4. The μ-opioid receptor agonist DAMGO produces excitation of mossy fiber responses evoked at low frequencies. *A*, Representative traces of mossy fiber responses in a single animal before (*a*) and after (*b*) application of 10 nmol DAMGO. μ-Opioid receptor–induced excitation was characterized by the appearance of synchronous spikes superimposed on field EPSPs without an apparent effect on the field EPSP slope. Calibration: 0.25 mV, 5 msec. *B*, Representative plot of the reversal of the excitatory effects of DAMGO by the μ-opioid receptor antagonist CTOP. Each point represents the peak magnitude (▼) and the field EPSP slope (□) of mossy fiber responses evoked in a single animal before and after application of DAMGO (10 nmol) and CTOP (3 nmol).

inducing mossy fiber LTP, produced a potentiation of mossy fiber responses when delivered 10 min following delivery of DAMGO (Fig. 5). This potentiation was equivalent in magnitude to mossy fiber LTP produced by 30–200 pulse bursts [mean percentage increase in mossy fiber responses following 30–200 pulses = 79.6 ± 10%; following delivery of a 15 pulse burst in the presence of DAMGO = 67.7 ± 10%; $F(1,12) = 7.49$, $p < 0.02$].

We addressed the similarity of potentiation produced by the DAMGO/15 pulse treatment to both DAMGO-induced potentiation and mossy fiber LTP. In contrast to the reversible potentiation observed following application of 1 nmol of DAMGO by itself, but similar to mossy fiber LTP produced by high-frequency stimulation, the potentiation produced by the combined DAMGO/15 pulse burst treatment was not reversed by application of CTOP (3 nmol) 1 hr later [mean percentage change in mossy fiber responses 1 hr following the DAMGO/15 pulse burst treatment = 67.7 ± 10%; 10–15 min following application of 3 nmol CTOP = 80.7 ± 8%; $F(1,4) = 0.989$, $p > 0.05$]. We also determined if potentiation produced by the combined DAMGO/15 pulse treatment attenuated subsequent induction of mossy fiber LTP. Fifty to sixty minutes following potentiation produced by 15 pulses delivered in the presence of DAMGO, a 200 pulse train was delivered to the mossy fibers (Fig. 6). Potentiation of mossy fiber responses by the 200 pulse burst was attenuated significantly by the DAMGO/15 pulse treatment [mean percentage change in mossy fiber field EPSP slopes following DAMGO/15 pulse treatment = 54.5 ± 12%; additional potentiation of mossy fiber responses produced by a 200 pulse burst = 9.3 ± 12%; $F(1,11) = 21.98$, $p < 0.001$], suggesting that the DAMGO/15 pulse treatment partially occluded tetanus-induced mossy fiber LTP.

In separate studies, we determined the minimal amount of high-frequency stimulation necessary for potentiating mossy fiber responses in the presence of 100 pmol DAMGO. Single pulses, paired pulses (50 msec interpulse interval delivered every 5 sec for 1 min), or bursts of five pulses at 100 Hz were ineffective in potentiating mossy fiber responses, whereas delivery of bursts of 10 pulses at 100 Hz in the presence of DAMGO reliably potentiated mossy fiber responses ($n = 3$). By contrast, bursts of 10–15 pulses delivered in the presence of lactated Ringer's were ineffective in potentiating mossy fiber responses ($n = 3$; Fig. 7).

Discussion

The present results indicate that the induction of mossy fiber LTP requires a burst of high-frequency stimulation composed of at least 30 pulses at stimulation frequencies of 100 Hz. In addition, the 30 pulse requirement appears to be threshold for maximal mossy fiber LTP induction, because the magnitude of mossy fiber LTP induced with 30, 50, or 200 pulses did not

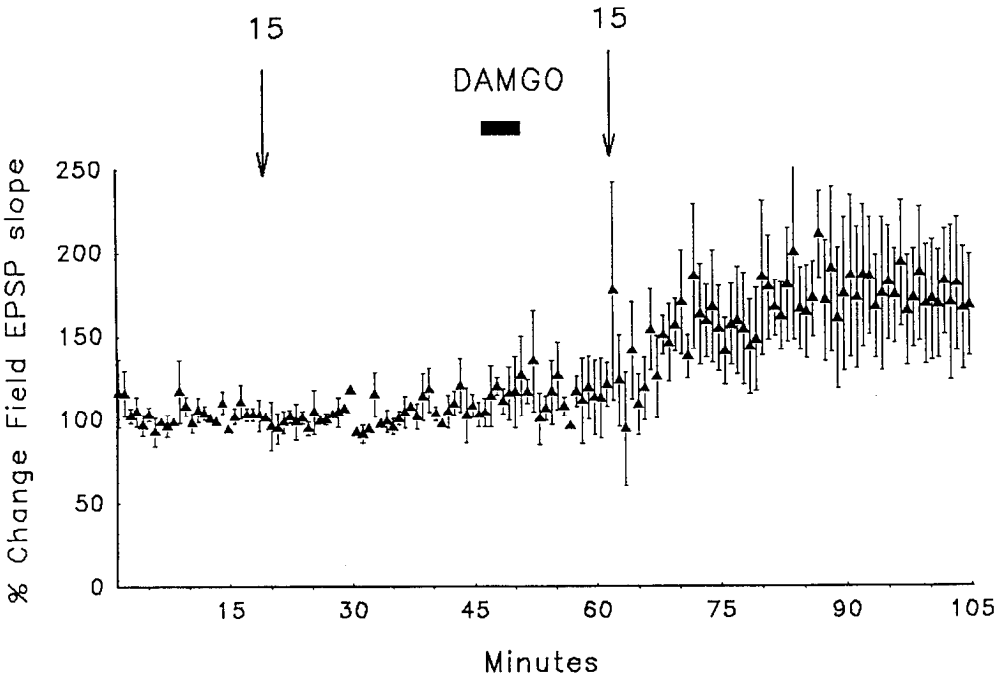

Figure 5. Summary of the effect of the μ-opioid receptor agonist DAMGO on potentiation produced by a single burst of 15 pulses. Each point represents the mean fractional percentage change (±SEM) of mossy fiber field EPSP slope magnitudes evoked once per minute (n = 4). A single 15 pulse train (100 Hz) did not potentiate mossy fiber responses. Application of DAMGO (100 pmol) also did not potentiate mossy fiber responses. However, potentiation was observed with a 15 pulse train when delivered in the presence of 100 pmol of DAMGO.

differ, and because delivery of trains subsequent to LTP induction resulted in little additional potentiation of mossy fiber responses. Thus sustained high-frequency stimulation is necessary for the induction of mossy fiber LTP, and delivery of stimulation at or above threshold induces maximal mossy fiber LTP in an all-or-none manner. These findings are contrasted by those reported for NMDA antagonist–sensitive LTP, which can be induced with trains composed of as few as 10 pulses (Barnes, 1979; Diamond et al., 1988), and which displays an incremental potentiation that summates in magnitude with subsequent high-frequency stimulation (see, e.g., Diamond et al., 1988; Castro et al., 1989; Bliss and Collingridge, 1993).

Previously, we demonstrated that the induction of mossy fiber–CA3 is blocked by antagonists selective for μ-, but not κ- or δ-, opioid receptors (Derrick et al., 1992). Previous studies by others also indicate that the calcium-dependent release of endogenous μ-opioid receptor ligands is observed during high-, but not low-, frequency mossy fiber stimulation (Wagner et al., 1990). These findings, taken with the present results demonstrating that the induction of mossy fiber LTP requires repetitive stimulation of the mossy fibers, together suggest that the release of μ-opioid receptor ligands as a result of repetitive mossy fiber synaptic activity is essential for the induction of mossy fiber LTP. If high-frequency mossy fiber stimulation is necessary for

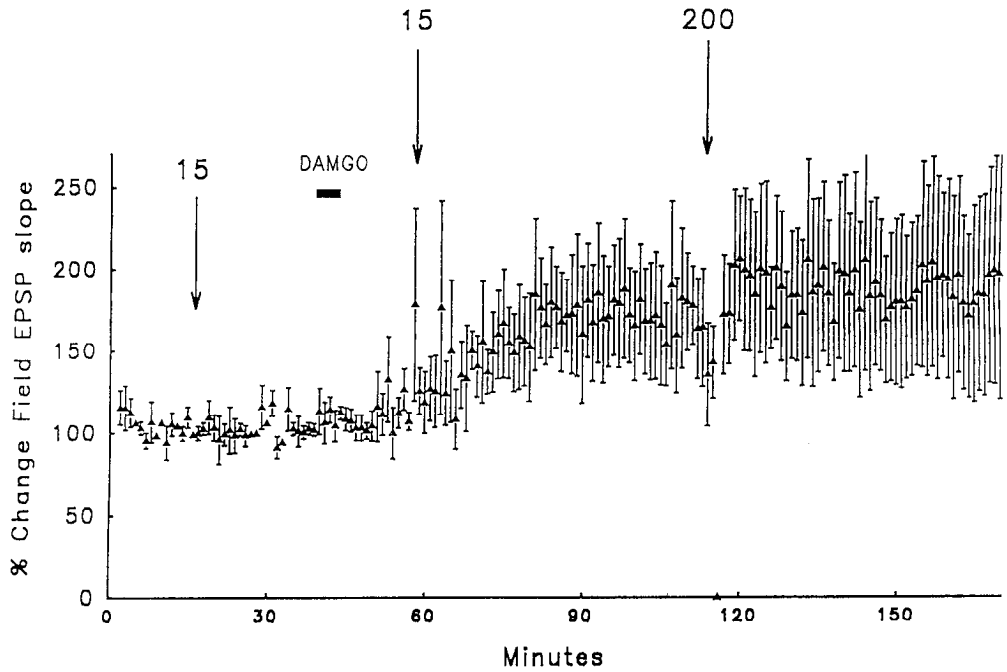

Figure 6. Mossy fiber LTP is occluded by potentiation produced by 15 pulses delivered in the presence of the μ-opioid receptor agonist DAMGO (100 pmol). Although 15 pulses alone did not potentiate mossy fiber responses, potentiation was observed following delivery of 15 pulses in the presence of DAMGO. This potentiation occluded subsequent mossy fiber LTP induced by a single 200 pulse burst (n = 4).

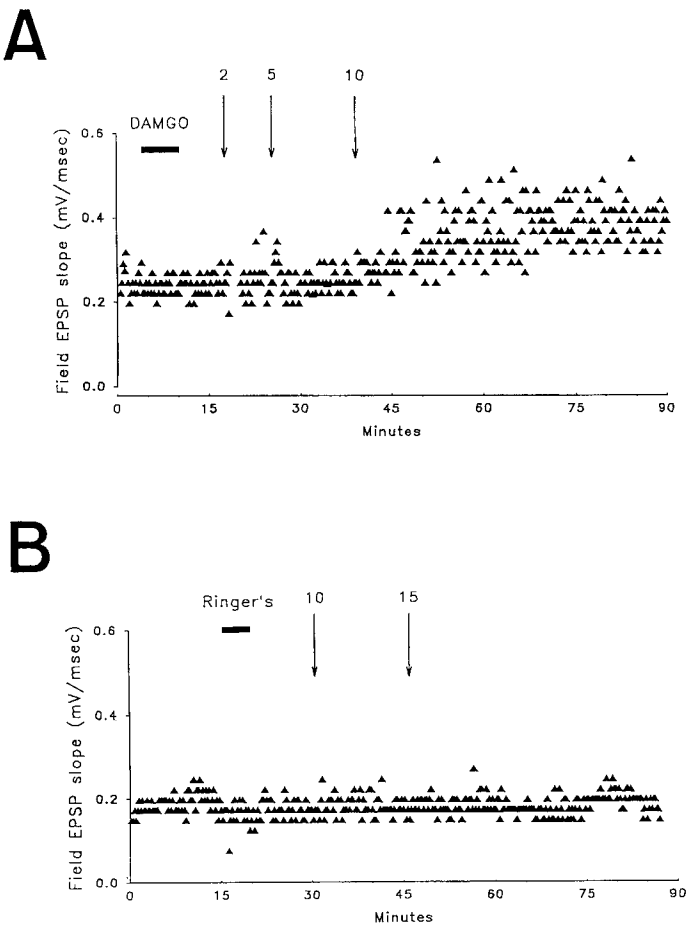

Figure 7. Representative plot of the effect of 100 Hz bursts of 2–15 pulses on mossy fiber responses in a single animal following delivery of a 100 pmol quantity of DAMGO (*A*) or lactated Ringer's vehicle (*B*). Although paired pulses (10 pairs delivered at a 0.25 Hz rate with interpulse intervals of 50 msec) or bursts of 5 pulses at 100 Hz were not effective in potentiating mossy fiber responses, burst of 10 pulses at 100 Hz in the presence of DAMGO potentiated mossy fiber responses. Potentiation of mossy fiber responses was not observed in the presence of the lactated Ringer's vehicle.

LTP induction solely to provide μ-opioid receptor activation, then it would be expected that exogenous application of a μ-opioid receptor agonist would preclude the need for high-frequency mossy fiber stimulation and result directly in the induction of mossy fiber LTP. Application of the μ-opioid receptor–selective agonist DAMGO (1 nmol) resulted in generation of population spikes without any apparent increase in the field EPSP slope, an effect that is consistent with a disinhibitory effect of this drug (Henriksen et al., 1988; Caudle and Chavkin, 1990). The excitatory effects of DAMGO were reversed by subsequent application of the μ-opioid receptor–selective antagonist CTOP. By contrast, increases in field EPSP slopes are observed with mossy fiber LTP, and established mossy fiber LTP is not reversed by subsequent application of opioid receptor antagonists (Derrick et al., 1991, 1992). Thus, the potentiation of mossy fiber responses by DAMGO appears to be qualitatively distinct from mossy fiber LTP, and the activation of μ-opioid receptors appears to be a necessary, but not a sufficient, condition for the induction of mossy fiber LTP.

Although DAMGO did not itself induce mossy fiber LTP, DAMGO reduced the number of high-frequency stimuli required to induce mossy fiber LTP. A 15 pulse burst at 100 Hz, which alone is ineffective in inducing LTP, induced potentiation of mossy fiber responses when delivered in the presence of DAMGO. The potentiation produced by the combined DAMGO/15 pulse treatment attained maximal amplitude after approximately 30 min, as is observed with stimulation-induced mossy fiber LTP *in vivo* (Derrick and Martinez, 1989; Yeckel and Berger, 1991; Derrick et al., 1992). Further, this potentiation was not reversed by CTOP, and partially occluded the generation of subsequent LTP induced by high-frequency stimulation. These results suggest that the potentiation of mossy fiber responses produced by the combined DAMGO/15 pulse treatment is identical to mossy fiber LTP. Because the amount of high-frequency stimulation necessary to induce mossy fiber LTP is reduced by exogenous application of μ-opioid receptor agonists, and because a μ-opioid receptor antagonist blocked the induction of mossy fiber LTP by brief mossy fiber trains, these results suggest that μ-opioid receptor activation, mediated presumably via endogenous opioid peptides released by high-frequency mossy fiber stimulation, is a principal factor underlying the requirement of sustained, high-frequency mossy fiber stimulation for the induction of mossy fiber LTP.

Recent studies suggest the involvement of another opioid receptor, the κ receptor, in the modulation of LTP (Wagner et al., 1993; Weisskopf et al., 1993). The study by Weisskopf et al. (1993) indicates that mossy fiber stimulation produces a depression of mossy fiber responses at adjacent, nontetanized, mossy fiber synapses (heterosynaptic depression). This effect is blocked by both selective κ-opioid receptor antagonists and naloxone, although these investigators failed to observe any attenuating effects of naloxone on mossy fiber LTP induction as reported previously by ourselves and others (Martin, 1983; Derrick and Martinez, 1989; Ishihara et al., 1990; Derrick et al., 1991; Williams and Johnston, 1992). This inhibitory effect is presumably mediated by the release of dynorphins, which are endogenous κ-opioid receptor ligands (Chavkin et al., 1982). Consistent with the findings of Weisskopf et al. (1993), our previous studies indicate that, in low quantities, dynorphin A (1-13) inhibits evoked mossy fiber–CA3, but not commissural–CA3, responses (Martinez and Derrick, 1989; Derrick, 1993). By contrast, we also observed that, in larger quantities, dynorphin A (1-13) produces excitatory effects that are blocked by μ-opioid receptor–selective antagonists, a finding that is consistent with other studies demonstrating the affinity of dynorphin A for μ-opioid receptors (Goldstein et al., 1981) and the μ-opioid receptor–mediated actions of dynorphin A in the hippocampus (Chavkin et al., 1985b; Self and Stein, 1992). Together, these data suggest that μ- and κ-opioid receptors mediate opposing processes at the mossy fiber synapse, and are consistent with previous studies suggesting the opposing actions of μ- and κ-opioid receptors at a variety of synaptic systems, including the mossy fibers (Bradley and Brooks, 1984; Iwama et al., 1986; Siggins et al., 1986; Janiri et al., 1988).

It is suggested that endogenous opioid peptides may facilitate the induction of LTP by attenuating GABAergic inhibition, an effect that can facilitate both postsynaptic depolarization and the induction of the NMDA receptor–dependent form of LTP (Wigstrom and Gustafsson, 1983). However in the present study DAMGO facilitated induction of mossy fiber LTP in a quantity (100 pmol) that did not produce excitatory effects. Because the excitatory effects of μ agonists on responses evoked in area CA3 are thought to be mediated by an opioid receptor–mediated

attenuation of GABAergic inhibition (Caudle and Chavkin, 1990), that μ agonists facilitate LTP induction in the absence of such effects suggests that μ-opioid receptor activation may contribute to mossy fiber LTP induction via actions that are independent of their actions on GABAergic inhibition. This suggestion is supported by a preliminary report (Williams and Johnston, 1992) indicating that the opioid receptor antagonist naloxone blocks the induction of mossy fiber LTP *in vitro* even when GABAergic inhibition is blocked by GABAergic antagonists. Although these findings support the speculation that opioid receptor activation may play an essential and direct role in mossy fiber LTP induction, rather than modulating LTP induction via their disinhibitory effects (Derrick and Martinez, 1989; Derrick et al., 1991, 1992), further studies will be essential for a convincing demonstration that μ-opioid receptors contribute to mossy fiber LTP induction via mechanisms that are independent of their disinhibitory actions.

Because activation of μ-opioid receptors is not sufficient to induce mossy fiber LTP, it appears that other factors in addition to μ-opioid receptor activation are required for mossy fiber LTP induction and are provided by brief mossy fiber trains. The additional factor(s) provided by high-frequency stimulation is not known, but there is evidence suggesting that brief high-frequency trains may be essential for mossy fiber LTP induction as a result of processes occurring at either pre- or postsynaptic sites. For instance, although extracellular calcium is thought to be essential for mossy fiber LTP induction (Higashima and Yamamoto, 1985), the availability of calcium at postsynaptic sites may not be (Zalutsky and Nicoll, 1990; Katsuki et al., 1991). Additionally, agents that attenuate mossy fiber PTP, a presynaptic process that is dependent on presynaptic calcium, also attenuate the induction of mossy fiber LTP (Kamiya, 1989; Derrick et al., 1992). Thus, the influx of sufficient quantities of calcium at presynaptic sites as a result of repetitive presynaptic activity may be an essential factor for the induction of mossy fiber LTP. Alternatively, other studies indicate that the induction of mossy fiber LTP depends on postsynaptic factors, such as postsynaptic depolarization and the availability of calcium postsynaptically (Williams and Johnston, 1989; Jaffe and Johnston, 1990). Thus, brief high-frequency stimulation may provide postsynaptic factors that, in conjunction with opioid receptor activation, may be essential for the induction of mossy fiber LTP. In the present study we found that, in the presence of DAMGO, bursts of 5–10 pulses are necessary to induce mossy fiber LTP. Similarly sized bursts are required for the induction of LTP at synapses sensitive to NMDA receptor antagonists (Diamond et al., 1988). This brief high-frequency activity is thought to be essential for attaining levels of postsynaptic depolarization necessary for the influx of calcium via postsynaptic NMDA receptor–dependent and/or voltage-dependent ionophores (Bliss and Lynch, 1988; Bliss and Collingridge, 1993). Similarly, such bursts could be necessary for attaining the levels of postsynaptic depolarization that, in conjunction with opioid receptor activation, are requisite for inducing mossy fiber LTP (Jaffe and Johnston, 1990; Martinez et al., 1990).

That sustained, high-frequency mossy fiber activity is essential for the induction of mossy fiber LTP may explain the apparent inability of mossy fiber synapses to display cooperative and associative LTP (Chattarji et al., 1989; Zalutsky and Nicoll, 1990, 1992). Mossy fiber LTP is not observed when single mossy fiber pulses are delivered in conjunction with either coactive afferents (Chattarji et al., 1989) or postsynaptic depolarization (Jaffe and Johnston, 1990; Zalutsky and Nicoll, 1990). In addition, the induction of mossy fiber LTP is suggested to be independent of the intensity of stimulation (Zalutsky and Nicoll, 1992), suggesting an absence of cooperativity (McNaughton et al., 1978) at this synapse. However, because the induction of mossy fiber LTP requires frequency-dependent activation of opioid receptors, it is possible that the inability of mossy fiber responses to display either associativity or cooperativity arises from the inability of the stimulation parameters used in previous studies to elicit the release of endogenous opioid peptides. Mossy fiber synapses may display associativity and cooperativity when mossy fiber synaptic activity is of sufficient duration to elicit the release of opioid peptides and the activation of μ-opioid receptors. In support of this suggestion, mossy fiber cooperativity is observed when sustained mossy fiber stimulation (Griffith, 1990), but not when trains of 10–15 pulses (Zalutsky and Nicoll, 1992), are used. Thus, mossy fiber LTP displays cooperativity when the requirement for high-frequency synaptic stimulation, and presumably opioid receptor activation, is met. It is not known if cooperativity at this synapse involves intensity-dependent factors acting at pre- or postsynaptic sites (see McNaughton et al., 1978). The involvement of intensity-dependent factors converging postsynaptically would indicate a potential for Hebb-like associative processes at this synapse (Jaffe and Johnston, 1990).

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