Opioid-Mediated Facilitation of Long-Term Depression in Rat Hippocampus

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ABSTRACT

Previous studies have demonstrated that opioid substances are often inhibitors of the γ-aminobutyric acid (GABA) transmitter system in the hippocampal formation, and that GABA-mediated inhibition is a potent modulator of synaptic plasticity. Exogenous opioid exposure on the induction of long-term depression (LTD) at excitatory synapses in the stratum radiatum. Exogenous application of a selective µ-opioid agonist resulted in a greater than 2-fold enhancement of LTD, whereas κ- and δ-agonists did not significantly affect LTD magnitude. Costimulation of the opioid peptide-containing stratum lacunosum-moleculare during LTD induction also resulted in a facilitation of LTD in the stratum radiatum, an effect prevented by prior administration of an opioid antagonist. These results suggest that both exogenously applied and endogenously released opioids can act to facilitate LTD of the Schaffer collateral input to CA1 pyramidal neurons.

The regulation of synaptic plasticity of excitatory synapses by GABAergic systems in the hippocampus has been well documented, and it is often suggested that changes in the level of GABAergic activity are a key factor in altering the susceptibility of a given synaptic pathway to a plasticity-evoking event (Wigstrom and Gustafsson, 1983; Abraham and Wickens, 1991; Wagner and Alger, 1995). These studies have typically used GABA antagonists in demonstrations of the influence of activation of GABA receptors on the induction of long-term potentiation (LTP) and long-term depression (LTD). However, to date, an in vivo source of such GABA antagonists has not been identified. In contrast, several endogenous neurochemicals can potentially act as antagonists of GABA via inhibition of GABA release (Thompson et al., 1993). The opioid peptides and their receptors comprise one such putative neurotransmitter system that is capable of acting as a physiologically relevant GABA antagonist.

In the CA1 region, LTD is commonly evoked via relatively long (5–15-min) episodes of low-frequency (1–3-Hz) stimulation (LFS; Dudek and Bear, 1992). In tissue obtained from adult animals (>40 days), most investigators report difficulty in inducing LTD, and this difficulty correlates developmentally with the postnatal maturation of the GABAergic circuitry within the hippocampus during 0 to 4 weeks (for review, see Wagner and Alger, 1996). NMDA receptor-dependent forms of both LTD and LTP have been extensively characterized in the CA1 (Bear and Abraham, 1996; Malenka and Nicoll, 1999), and the induction of both are facilitated in the presence of GABAA receptor antagonists, suggesting that endogenous GABAergic systems normally act to regulate plasticity at glutamatergic synapses. Therefore, the actions of neuromodulators on GABA neurons and terminals are likely to be crucial in determining the impact of potential plasticity-invoking stimulus events. In this report, we have examined the modulatory effects of opioid exposure on the subsequent induction of LTD in rat hippocampal slices via 1-Hz stimulation. The actions of both exogenously applied opiates and endogenously released opioids were assessed. Opiates capable of activating µ-opioid receptors were able to significantly increase the magnitude of LTD greater than 2-fold, whereas δ- and κ-selective agents did not have such facilitatory effects. We conclude that µ-opioid receptor activation, possibly through inhibition of GABAergic neurons, results in a significant enhancement of LTD evoked following low-frequency stimulation in the CA1 region of the rat hippocampal formation.

Materials and Methods

Extracellular Electrophysiology. Freshly prepared transverse hippocampal slices (500 µm) were obtained from mature (40–70-day-old) adult animals. All experiments were performed within 3 hr of slicing. The slices were maintained at 34°C in a humidified atmosphere of 95% O2 and 5% CO2. The superfusion fluid consisted of (in mM): NaCl 127, KCl 5, CaCl2 2.5, MgCl2 1, NaHCO3 24, NaH2PO4 1, and glucose 10. The slices were perfused at a rate of 1 mL/min. For recordings, the effluent was collected from the recording chamber and the extracellular field potentials were amplified, digitized, and recorded on a computer. The stimulation intensity was adjusted to evoke a submaximal field excitatory postsynaptic potential (fEPSP) response. Stimulation was delivered as a 10-Hz train of square waves (80 V, 0.1 ms, 0.5 sec). The initial fEPSP amplitude was recorded, and the same stimulus intensity was maintained throughout the experiment. The slice was then exposed to 100 nM of DAMGO (a µ-selective agonist) for 20 min, followed by 20 min of washout. After the washout was completed, the LTD magnitude was recorded for 20 min. The fEPSP response was recorded in the absence of DAMGO for 20 min. The duration of each experiment was approximately 1 h. The field potentials were expressed as a percentage of the initial fEPSP amplitude. The data were analyzed using paired t-tests for within-group comparisons and unpaired t-tests for comparisons between groups. The p-values were calculated for each experiment. The data are presented as mean ± SEM, and the significance level was set at p < 0.05.

ABBREVIATIONS: GABA, γ-aminobutyric acid; LTP, long-term potentiation; LTD, long-term depression; LFS, low-frequency stimulation; NMDA, N-methyl-D-aspartate; s. radiatum, stratum radiatum; s. lac-mol., stratum lacunosum-moleculare; fEPSP, field excitatory postsynaptic potential; SC, Schaffer collateral; DAMGO, [D-ala²,N-Me-Phe³,Gly⁵-ol]-enkephalin; TA, temporoammonic; NMDAR, NMDA receptor; APV, α,-2-amino-5-phosphonovaleric acid.
old) Sprague-Dawley rats anesthetized (Halothane) before decapitation. The CA3 region was surgically removed immediately after slice dissection. Slices were submerged in a recording chamber and perfused continuously with saline saturated with 95% O$_2$-5%CO$_2$ at approximately 1 ml/min. The recording chamber and perfusion saline were warmed to 30°C for the duration of the experiment, and the slices were incubated for at least 1.5 h in the chamber before an experiment was begun. The saline contained 120 mM NaCl, 3 mM KCl, 1.5 mM MgCl$_2$, 1 mM Na$_2$HPO$_4$, 2.5 mM CaCl$_2$, 26 mM NaHCO$_3$, and 10 mM glucose. Extracellular recording electrodes (1–2-μm tip) filled with 200 mM NaCl were placed in the s. radiatum of CA1. Field EPSP population responses were evoked with a bipolar stimulating electrode (Kopf Instruments, Tunjunga, CA) placed on either the CA3 or the subicular side of the recording electrode in the s. radiatum. Stimulation parameters consisted of single square waves of 40 to 90 μA (150 μA for lac-mol. stimulation) of 300-μs duration. Data were digitized at 10 kHz and analyzed with pCLAMP 7 software (Axon Instruments, Foster City, CA). The initial slope of the population EPSP was measured by fitting a straight line to the first millisecond of the EPSP immediately following the fiber volley. Stimulus-response curves were performed at the beginning of each experiment. Pulses of an intensity that gave 40 to 60% of the maximum response were given at a frequency of 0.05 Hz for the remainder of the experiment. All stimulation protocols were performed at the test pulse intensity, and when two synaptic pathways were monitored, their independence was evaluated as previously described (Wagner and Alger, 1995). The 1-Hz LFS protocol consisted of a 10-min period of 1-Hz stimulation that was repeated after a 10-min interval. For clarity, the responses during LFS are not illustrated.

**Quantification of Synaptic Plasticity.** LTD was quantified 25 to 30 min after the completion of the LFS protocol by averaging the EPSP slopes from 15 consecutive responses at baseline frequency and dividing this value by the average of the 15 EPSP slopes from 5 min before beginning LFS. Unless otherwise noted, the n values reported represent slices taken from different animals for a given experimental group (e.g., n = 6 is six slices from six different animals). All drugs were obtained from Sigma (St. Louis, MO).

**Results**

**LTD Is Increased in the Presence of a μ-Agonist.** The fEPSP was monitored in s. radiatum of the CA1 region following stimulation of the Schaffer collateral (SC) input. An LFS protocol consisting of two episodes of 1-Hz stimulation/600 pulses separated by a 10-min interval was used in the attempt to elicit LTD. As shown in Fig. 1A, 1-Hz stimulation had a relatively small depressive effect on the baseline response in slices obtained from 40- to 70-day-old rats (90 ± 3%, n = 7). In contrast, the depression was significantly enhanced (77 ± 3%, n = 10 slices from seven animals) in the presence of the μ-opiate agonist [d-Ala$_2$N-Me-Phe$_4$, Gly$_8$-ol]-enkephalin (DAMGO; Fig. 1B). DAMGO (10 μM) was administered 30 min before the initiation of LFS.

This enhancement of LFS-induced depression was dose-dependent; following 1-Hz stimulation in the presence of 0.3, 1, and 3 μM DAMGO, fEPSP responses were 88, 84, and 82% of baseline, respectively. This trend is illustrated in Fig. 2A, where the facilitation of LTD magnitude (relative to the 10% decrease observed in the control group of slices from Fig. 1A) is plotted versus the concentration of DAMGO present.

**DAMGO Effects Are Naloxone-Sensitive.** When naloxone (10 μM) was administered to the slices 30 min before opiate exposure, the facilitatory effect of DAMGO (10 μM) was significantly attenuated compared with that of DAMGO alone (DAM/Nal, 88 ± 1%, n = 9 slices from six animals; Fig. 2B). Naloxone by itself displayed no inhibitory effect on LTD magnitude compared with the control group (naloxone, 89 ± 3%, n = 14 slices from eight animals; Fig. 2B), indicating that the antagonist effect was not due to merely masking the DAMGO-mediated facilitation. These results demonstrate that the enhancement of LTD following LFS in the presence of DAMGO is likely mediated through the activation of a naloxone-sensitive opioid receptor.

**δ- and κ-Agonists Do Not Enhance LTD.** Although DAMGO has been characterized as a μ-selective opioid agonist (Handa et al., 1981), it is possible that at the higher concentrations used in this study, other types of opiate receptors could be activated. To further characterize the mechanism of the DAMGO effect, putative subtype-selective opiate agonists were tested (Fig. 2C). Neither the δ-selective agonist [d-Pen$_2$,d-Pen$_2$]-enkephalin (Mosberg et al., 1983) nor the κ-selective agonist (+)-(5a,7a,8b)-3,4-dichloro-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide mesylate (U69,593; Lahti et al., 1985) was effective in significantly altering the LTD observed following LFS (96 ± 7%).
LTD in slices obtained from mature rats (Wagner and Alger, 1995), and since opiate actions in the hippocampus have often been shown to be mediated via disinhibition, we coadministered DAMGO (10 μM) and bicuculline (10 μM) and tested the effectiveness of LFS under these conditions. As seen in Fig. 2C, the combination of the μ-agonist and the GABA<sub>A</sub> antagonist was no more effective in facilitating LTD than DAMGO alone (78 ± 5%, n = 6 slices; Fig. 2C). The lack of additivity indicates that DAMGO and bicuculline may be acting via a common mechanism (i.e., inhibition of the GABAergic input).

**Costimulation of the Opioid-Containing S. Lacunom-Molecule Enhances LTD.** The results described in Figs. 1 and 2 suggest that activation of μ-opioid receptors enhances the magnitude of LFS-induced LTD in the s. radiatum of CA1. A source of endogenous ligands for these μ-receptors is known to be present in s. lac-mol. of CA1, where an enkephalin-containing population of interneurons resides near the s. radiatum border (Gall et al., 1981). In an attempt to stimulate the release of endogenous opioid peptides, we applied LFS to both s. radiatum and s. lac-mol. at an interstimulus interval of 500 ms (i.e., 1 Hz at each site, alternating pulses). Costimulation of the SC pathway (Fig. 3A, S1) and the temporomammillary (TA) pathway (Fig. 3A, S2) in this manner resulted in significant depression of the s. radiatum fEPSP (77 ± 3%, n = 9 slices from eight animals; Fig. 3A) of a magnitude identical to that observed in the presence of 10 μM DAMGO (compare with Fig. 1B). In contrast, costimulation of two independent Schaffer collateral inputs (Fig. 3B, S1 and S2) did not result in a significantly enhanced depression (88 ± 3%, n = 7 slices from four animals; Fig. 3B) compared with LFS of a single input (Fig. 1A). Thus, the enhancement of LTD following s. lac-mol. stimulation is site-specific, and not attributable to merely an increased number of stimulus pulses being delivered to the slice during LFS.

**LTD Resulting from Lac-Mol. Costimulation Is Opiate- and NMDA Receptor-Dependent.** One site-specific effect of s. lac-mol. stimulation would potentially involve the release of proenkephalin-derived peptides from endogenous stores (Gall et al., 1981). We tested this possibility with the nonselective opiate antagonist naloxone. When naloxone (10 μM) was administered to the slices 30 min before lac-mol. costimulation, the facilitatory effect was completely attenuated compared with that of costimulation alone (94 ± 4%, n = 6 slices from five animals; Fig. 4A). This result indicates that the enhancement of LTD following lac-mol. costimulation is likely to be mediated through the activation of a naloxone-sensitive opioid receptor. In addition, the enhanced LTD resulting from lac-mol. costimulation is dependent upon the activation of NMDA receptors, because LFS was ineffective in the presence of the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV; 100 μM, 93 ± 4%, n = 6 slices from four animals; Fig. 4B).

**Discussion**

A primary result of this study is the determination that both exogenously applied opiates and endogenously released opioids act to modulate synaptic plasticity (i.e., NMDA-dependent LTD) in the CA1 region of the rat hippocampus. Although both opioid peptides (Gall et al., 1981) and opioid binding sites (McLean et al., 1987) have been localized in the
CA1, no examples of opioid actions following evoked release from endogenous stores in this region have been reported. In contrast, the effects of applied opiates have been well characterized. Exogenous application results in an indirect excitation of pyramidal cells (Zieglgansberger et al., 1979), presumably via hyperpolarization of GABAergic interneurons (Madison and Nicoll, 1988) and/or inhibition of GABA release from their terminals (Cohen et al., 1992). This inhibition of inhibitory interneurons (i.e., disinhibition) forms the rationale for the atypical excitatory effects of opiate application on principal neurons in the hippocampus relative to other brain areas (Nicoll et al., 1977). Our results indicating that coadministration of a GABA antagonist along with the μ-agonist yields no additional facilitatory effect on LTD magnitude is consistent with a disinhibitory mechanism underlying the opioid effects described herein. For this NMDAR-dependent LTD induced via LFS stimulation, either postsynaptic blockade of GABA\(_A\) receptors or presynaptic inhibition of GABA release would be expected to enhance the NMDA receptor activation during LFS that is required for the induction of LTD in the CA1. The question as to how endogenous opioids affect either non-NMDAR-dependent LTD (cf. Bolshakov and Siegelbaum, 1994), or LTD induced by other patterns of

![Fig. 3](image-url) Costimulation in the s. lac-mol. during LFS enhances LTD magnitude. A and B, as in Fig. 1, the data are normalized to the 5-min period immediately preceding the first LFS episode, and the period from 25 to 30 min following the second LFS was used to evaluate LTD magnitude. Insets are the averages of 15 field potential sweeps (horizontal bar is 50 ms) resulting from the two stimulation sites before the initiation of LFS (vertical bar is 1.5 mV) in representative experiments. A, summary plot \((n = 9)\) of normalized fEPSP measurements from the S1 (s. radiatum) input. LFS was performed as in Fig. 1, except a second stimulation site (S2, s. lac-mol.) was also stimulated 500 ms after S1, at a frequency of 1 Hz (i.e., costimulation). B, summary plot \((n = 7)\) of normalized fEPSP measurements from the S1 (s. radiatum) input. Costimulation was performed as in A, except that the second stimulation site (S2) was located in s. radiatum, not s. lac-mol.

![Fig. 4](image-url) Enhancement of LTD by s. lac-mol. costimulation is site-specific, naloxone-sensitive, and APV-sensitive. A and B, as in Fig. 3A, the data are normalized to the 5-min period immediately preceding the first LFS episode, and the period from 25 to 30 min following the second LFS was used to evaluate LTD magnitude. Costimulation of s. radiatum and s. lac-mol. inputs was performed. A, summary plot \((n = 6)\) of normalized fEPSP measurements from the s. radiatum input. Naloxone (10 μM) was administered 30 min before the initiation of costimulation. B, summary plot \((n = 6)\) of normalized fEPSP measurements from the S1 (s. radiatum) input. APV (100 μM) was administered 30 min before the initiation of costimulation. C, summary quantitation from the experiments performed as in Fig. 3. The dotted line across the bar graph indicates the amount of depression observed in control slices following LFS. Bars are the mean ± S.E.M. **A significant enhancement of depression compared with both the control group and the other data groups of the graph \((p < 0.01, \text{ANOVA followed by Student-Newman-Keuls post hoc tests})\).
conditioning stimulation (cf. Thiels et al., 1994), in this area of the hippocampus remains open.

Recent anatomical studies using electron microscopy have confirmed that μ-opioid receptors are found almost exclusively on GABAergic neurons in the hippocampus (Drake and Milner, 1999), and that the lacunosum-moleculare enkephalinergic interneurons exclusively innervate other GABAergic neurons (Blasco-Ibanez et al., 1998). Using such information (also see Freund and Buzsaki, 1996), Fig. 5 illustrates a schematic of the relevant components of a hypothetical CA1 circuit that is consistent with both our current results and the previously mentioned anatomical data. In this scenario, stimulation of the excitatory TA terminals feed-forward activates lac-mol. interneurons near the s. radiatum border. These lac-mol. interneurons release enkephalin that can act to inhibit μ-opioid receptor-expressing interneurons located in stratum radiatum that normally are activated by SC terminals in this layer. Thus, stimulation of s. lacunosum-moleculare during 1-Hz costimulation can result in disinhibition mediated by endogenous opioid release, whereas costimulation with a second SC input is less likely to activate enkephalinergic cells, and would also recruit additional concurrent feed-forward inhibition of pyramidal cells by s. radiatum interneurons. This illustration is one simplified example that outlines the potential circuit interactions underlying our results; of course, many other less parsimonious possibilities are also viable, given the already extensive (but probably incomplete) list of interneuron types present throughout all strata of CA1 (Freund and Buzsaki, 1996).

The TA pathway has been the focus of several studies that have characterized the excitatory input to the distal apical dendrites of CA1 pyramidal neurons (Doller and Weight, 1982; Cobert and Levy, 1992; Leung et al., 1995). In considering the direct excitatory effect on pyramidal cells, it has been suggested that this direct input from cortex to s. lac-mol. may serve as the first of a two-phase feed-forward excitation of the CA1 (Yeckel and Berger, 1990). Other studies have pointed toward a strong, polysynaptic, inhibitory influence on CA1 excitability resulting from TA activation (Empson and Heinemann, 1995). Despite disparate views regarding the net influence of the TA input on CA1 excitability (Soltesz and Jones, 1995), it is generally acknowledged that the existence of a direct cortical input to CA1 is likely to have important modulatory influences on synaptic information flow through the region. For example, recent studies have indicated that an inhibitory influence of TA activation can act to inhibit spike firing and LTP of CA1 neurons in response to SC stimulation (Levy et al., 1998; Dvorak-Carbone and Schuman, 1999). Our results demonstrating an opioid-dependent disinhibition serve to describe another mechanism by which such interlaminar modulation of synaptic processing can occur in the CA1. The feed-forward, polysynaptic inhibition of s. radiatum interneurons by TA activation of s. lac-mol. enkephalinergic interneurons would potentially have a large impact on synaptic input to the population of CA1 pyramidal cells. Interneurons often make numerous contacts with many pyramidal cells, and SC input in the s. radiatum is very effective in driving the firing of pyramidal cells. Therefore, the modulation of s. radiatum interneuron activity via the TA pathway would presumably have great influence on the output of CA1 neurons.

There are currently few examples of modulation of LTD by endogenously released neurotransmitters in the CA1 region. The facilitatory effects of GABA<sub>A</sub> and A<sub>1</sub> receptor antagonists on LTD indicate that both GABA (Kerr and Abraham, 1995; Wagner and Alger, 1995) and adenosine (Kemp and Bashir, 1997) released during LFS of the s. radiatum are likely to be important contributors in the determination of the extent of activity-dependent synaptic depression. Opioid peptides can now be added to such a group of modulators of LFS-induced plasticity, with the additional feature that the modulation would appear to be interlaminar in nature because it involves a cortical input to the CA1 in s. lac-mol. that is distinct from the CA3 input in s. radiatum. Opioid-mediated modulation of synaptic plasticity of the Schaffer collateral input comprises a unique mechanism by which the temporosommonic pathway can exert its influence on information processing through the CA1 region of the hippocampus.

In summary, our findings suggest that activation of μ-opioid receptors in the CA1 region of the hippocampus can be a key event in the determination of LTD magnitude following the administration of LFS in s. radiatum. Interneurons that can be activated by the TA input are known to contain an endogenous ligand (i.e., enkephalin) for these receptors, and these interneurons have been anatomically identified as being likely to serve as disinhibitors of pyramidal cells, thereby facilitating the induction of NMDA receptor-dependent forms of synaptic plasticity. Thus, in addition to the CA3 (Martin, 1983) and the dentate gyrus (Bramham et al., 1988) regions, opioid actions in the CA1 must also be considered when evaluating the systemic effects of opioid agonists/antagonists on learning tasks associated with hippocampal function.

References
Bramham CR, Errington ML and Bliss TVP (1988) Naloxone blocks the induction of...


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