Effects of μ-Opioid Receptor Modulation on GABA$_B$ Receptor Synaptic Function in Hippocampal CA1

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McQuiston AR. Effects of μ-opioid receptor modulation on GABA$_B$ receptor synaptic function in hippocampal CA1. J Neurophysiol 97: 2301–2311, 2007. First published January 10, 2007; doi:10.1152/jn.01179.2006. Activation of μ-opioid receptors (MORs) alters information coding, synaptic plasticity, and spatial memory in hippocampal CA1. In CA1, MORs act by inhibiting GABA release onto both GABA$_A$ and GABA$_B$ receptors exclusively. MOR activation can facilitate excitatory inputs in CA1 dendritic layers by inhibiting synaptic activation of GABA$_A$ receptors. In this study, we use voltage-sensitive dye imaging to show that MOR activation by the MOR agonist DAMGO suppressed GABA$_B$ inhibitory postsynaptic potentials in all layers of CA1. When stimulating excitatory input in stratum oriens (SO), stratum radiatum (SR), or stratum lacunosum-moleculare (SLM) with five pulses at 20 Hz in the presence of bicuculline (50 μM), DAMGO (1 μM) was most effective at increasing the amplitude of the last excitatory event. This effect was reversed by the MOR antagonist CTOP (1 μM) and occluded by the GABAB receptor agonist CGP 55845 (10 μM). DAMGO was less effective at increasing the amplitude of later excitatory events compared with the effect of CGP 55845. DAMGO was relatively ineffective at increasing the amplitude of excitatory inputs in SLM but had significantly greater effects on excitatory events as they propagated to stratum pyramidale (SP). When stimulating in SR, DAMGO was least effective at increasing excitatory amplitudes in SLM and most effective in SP and SO. Finally, DAMGO was equally effective at increasing excitatory activity amplitudes in all layers of CA1 after stimulating in SO. Therefore MOR suppresses GABA$_B$ synaptic hyperpolarizations in all layers of CA1 and most effectively facilitates excitatory activity in CA1 output layers.

INTRODUCTION

μ-Opioid receptor (MOR) activation can modulate spatial memory formation and synaptic plasticity in area CA1 of the hippocampus (Mansour et al. 1997, 1999; Pourmotabbed et al. 1998; Pu et al. 2002; Wagner et al. 2001). In addition to synaptic plasticity, MOR activation disrupted synchronous oscillations among populations of CA1 pyramidal cells, which may be important in the coding of information (Faulkner et al. 1998, 1999; Whittington et al. 1998). Although MORs can acutely and chronically affect synaptic plasticity, exactly how MOR modulation of hippocampal neurons and synapses can change network properties is not completely understood.

In CA1, MORs have been shown to be largely concentrated in the pyramidal cell layer [stratum pyramidale (SP)] with smaller amounts observed in the distal apical dendritic region [the stratum lacunosum-moleculare (SLM)] (Arvidsson et al. 1995; Atweh and Kuhar 1977; Crain et al. 1986; Ding et al. 1996; Herkenham and Pert 1980; Mansour et al. 1987, 1994, 1995; McLean et al. 1987) where they were located on the axons, terminals, dendrites, and somata of GABAergic inhibitory interneurons exclusively (Bausch et al. 1995; Drake and Milner 1999, 2002; Kaylyuzhny and Wessendorf 1997). MORs were most frequently found on interneurons that innervate pyramidal cell bodies and less on interneurons that innervate the distal apical dendrites of CA1 pyramidal cells (Drake and Milner 2002). Consistent with the anatomical studies, activation of MORs was shown to hyperpolarize CA1 inhibitory interneurons (Madison and Nicoll 1988; Svoboda and Lupica 1998; Svoboda et al. 1999; Wimpey and Chavkin 1991) and presynaptically inhibit GABAergic inhibitory postsynaptic potentials (IPSPs) onto CA1 pyramidal neurons (Capogna et al. 1993; Cohen et al. 1992; Lupica 1995; Lupica et al. 1992; Masukawa and Prince 1982; Nicoll et al. 1980; Rekling 1993; Swearengen and Chavkin 1989; Wimpey et al. 1990). Moreover, interneurons in stratum oriens (SO) that project to peri-somatic regions of pyramidal cells were almost all sensitive to MOR activation, whereas less than half of interneurons innervating the various dendritic regions were sensitive to the activation of MOR (Svoboda et al. 1999).

These anatomical and electrophysiological studies suggest that the predominant outcome of MOR activation in hippocampal CA1 would be to disinhibit the output of CA1 pyramidal cells with a much smaller effect on the inputs or synaptic integration within the dendrites. However, even with a small density of MORs located in CA1 dendritic fields, MOR activation of these interneurons could potentially exert a powerful effect on the network (Larkum et al. 1999; Miles et al. 1996), influencing synaptic input and dendritic integration within CA1 pyramidal cells. In support of a mechanism of action for MOR activation within the dendrites of CA1 pyramidal cells, voltage-sensitive dye (VSD) studies showed that MOR activation can enhance paired-pulse excitatory activity within the dendrites of CA1 pyramidal neurons and that this enhancement was the result of an inhibition of γ-aminobutyric acid type A (GABA$_A$) IPSPs (McQuiston and Saggau 2003). However, MOR activation in CA1 pyramidal cells was also shown to inhibit GABA$_B$ IPSPs (Lupica and Dunwiddie 1991), but the site of action for MOR effects on GABA$_B$ synaptic inhibition along the somatodendritic domain of CA1 pyramidal cells has not been examined. Therefore in an effort to determine the anatomical location of MOR suppression of GABA$_B$ synaptic function in hippocampal CA1, we isolated GABA$_B$ synaptic function from GABA$_A$ synaptic activity with bicuculline (50
µM) and used VSD imaging to simultaneously measure changes in inhibitory and excitatory synaptic events in all layers of CA1 after the application of the MOR agonist DAMGO.

**METHODS**

**Preparation of hippocampal slices and staining with voltage-sensitive dye**

Male Sprague–Dawley rats (42 to 60 days old) were deeply anesthetized with ketamine and xylazine, transcardially perfused with ice-cold saline [consisting of (in mM): sucrose 230, KCl 2.5, CaCl 1.2, MgCl 2, NaHPO 4, NaHCO 3, and glucose 10], and killed by decapitation in adherence with an approved Virginia Commonwealth University IACUC protocol. The brain was removed and hemisected and horizontal slices containing the midtemporal hippocampus were cut at 350 µm on a Vibratome 3000 (Ted Pella, Redding CA). Sections were incubated in a holding chamber kept at 32°C for 30 min and then allowed to return to room temperature. The holding chamber solution consisted of (in mM): NaCl 125, KCl 3.0, CaCl 2, MgCl 2, NaHPO 4, NaHCO 3, and glucose 10. Slices were stained for 30 to 60 min with the voltage-sensitive dye (VSD) NK3630 (0.02 to 0.05 mg/ml) before experimentation (Jin et al. 2002).

**VSD imaging and electrophysiology**

After staining with NK3630, slices were submerged and continuously perfused in a glass-bottom recording chamber with warmed saline (33–35°C) consisting of (in mM): NaCl 125, KCl 3.0, CaCl 1.2, MgCl 2, NaHPO 4, NaHCO 3, and glucose 25 bubbled with 95% O 2-5% CO 2. The recording chamber was mounted on a fixed stage under an Olympus BX51WI microscope equipped with differential interference contrast optics. The image of the slice was collected using transmitted near-infrared light (>775 nm) with a ×20 (0.95 NA) water-immersion objective. The image was captured (Foresight I-50 frame grabber) with a Dage-MTI NC-70 Newvicon tube camera or DAGE-MTI IR1000 CCD camera, both with contrast enhancement.

For VSD absorbance measurements, slices were illuminated with a tungsten–halogen 100-W lamp passed through a band-pass filter (705 ± 30 nm, Chroma Technology, Rockingham, VT). The transmitted light was collected with a Wutech H-4691V photodiode array that is part of the Redshirthading integrated Neureplex II imaging system, mounted on the front port of the Olympus BX51WI microscope. The data were acquired, displayed, and analyzed with Neuroplex software.

To evoke synaptic electrical activity in hippocampal CA1, bipolar tungsten stimulating electrodes (about 100 kΩ, FHC) were placed in either SO, stratum radiatum (SR), or SLM of CA1 to stimulate select groups of afferents. Electrical current was delivered (100 µS, 10–100 µA) by a stimulation isolation unit (NL800, Digitimer, Hertfordshire, UK) to produce excitatory postsynaptic potential (EPSP)–like events or IPSPs. Individual photodiodes of the array that form a line perpendicular to the signal and comparing amplitudes of subsequent excitatory events resulting from a train of stimuli. Otherwise, a Student–Newman–Keuls test was used when comparing groups with no obvious order or a Bonferroni correction was applied when comparing only two groups. All reported values are means ± SE.

**Statistics and data analysis**

The data were analyzed using WCP software for the electrophysiological measurements and Neureplex II software for the VSD recordings. Statistics were performed using GraphPad Instat (GraphPad Software, San Diego, CA). For paired measurements, statistical significances (P < 0.05) were determined by paired t-test and repeated-measures ANOVA; for unpaired measurements one-way ANOVA was used. In some analyses, post hoc tests were performed to determine differences between individual groups. A test for linear trend was used when comparing different layer responses to a propagating signal and comparing amplitudes of subsequent excitatory events resulting from a train of stimuli. Otherwise, a Student–Newman–Keuls test was used when comparing groups with no obvious order or a Bonferroni correction was applied when comparing only two groups. All reported values are means ± SE.

**Chemicals**

All chemicals were purchased from VWR unless otherwise indicated. NK3630 was obtained from Leslie M. Loew (University of Connecticut, Farmington, CT) or Hayashibara (Okayama, Japan). Bicuculline methochloride (BIC), 6,7-dinitroquinoxaline-2,3-dione (DNQX), D-2-amino-5-phosphonovaleric acid (APV), (2S)-3-$\text{[1'-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl]phenylmethyl}$ phosphinic acid (CGP 55845), D-Ala2, NMe-Phe4, Gly-ol5-enkephalin (DAMGO), and D-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH 2 (CTOP) were all purchased from Tocris Bioscience (Ellisville, MO).

**RESULTS**

This study investigated the effect of MOR activation on the synaptic activation of GABA B receptors and its subsequent
effect on the propagation of excitatory activity in hippocampal CA1. To do this, voltage-sensitive dye (VSD) imaging was used to measure electrical activity occurring simultaneously throughout the hippocampal CA1 network. This technique was shown to reliably measure electrical responses in populations of neurons often with a better signal-to-noise ratio than electrical field potentials (Jin et al. 2002). More specifically, VSD imaging could reliably measure small electrically evoked excitatory responses in neocortical brain slices that were undetectable by field potential methods. Furthermore, VSD signals and field potential measurements frequently showed similar waveform kinetics (Jin et al. 2002). In addition to field potentials, VSD signals in CA1 pyramidal neuron apical dendrites showed the same kinetics as that of intracellular electrical signals measured using dendritic whole cell patch clamping (Ang et al. 2005). Thus, VSD imaging was shown to accurately record electrical activity from populations of neurons in different regions of the CNS. In addition to VSD imaging, in some experiments simultaneous whole cell patch clamp or field excitatory postsynaptic potentials (fEPSPs) were recorded for comparison. Electrical responses were evoked by five pulses at 20 Hz in the SO, SR, or SLM. A train of stimuli was used to increase the likelihood that GABA was released from interneurons with both high and low probabilities of release (Gupta et al. 2000; Reyes et al. 1998). Trains of stimuli were also used to measure the effect of MOR activation on pyramidal cell excitability after a delay because the effect of GABAB receptors may require a few hundred milliseconds to reach their peak activation after GABA release. To isolate the effects of GABAB receptor activation from GABAA receptor activation, experiments were conducted in the presence of the GABAA receptor antagonist bicuculline (BIC, 50 μM). To prevent excitotoxicity and seizure activity in the slices, a cut was made to sever the connection between CA3 and CA1 in all experiments.

**MOR activation increases excitatory activity amplitudes**

As previously observed (McQuiston and Saggau 2003), activation of MORs (DAMGO, 1 μM) increased EPSPs in hippocampal CA1 SLM and facilitated the propagation of the

*Fig. 1. Activation of μ-opioid receptors increased excitatory postsynaptic activity. A: image of voltage-sensitive dye (VSD; NK3630) signals overlying hippocampal slice. Signals were produced by stimulating afferents in stratum lacunosum-moleculare (SLM) of hippocampal CA1: yellow arrow, tungsten-stimulating electrode (5 pulses, 20 Hz); blue arrow, whole cell patch electrode. Six adjacent photodiode VSD signals were spatially averaged for each anatomical layer. Photodiodes chosen for this experiment were: dark blue, SLM; orange, stratum radiatum (SR); light blue, stratum pyramidale (SP); red, stratum oriens (SO). Averages are shown in B. B: stimulation in SLM of CA1 produced VSD signals in each anatomical layer of CA1. VSD signals: black, Control; blue, μ-opioid receptor (MOR) agonist [D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin (DAMGO, 1 μM); orange, γ-aminobutyric acid type B (GABAB) receptor antagonist (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl][phenylmethyl]phosphinic acid (CGP 55845, 10 μM); green, CGP 55845 (10 μM) + GABAA receptor antagonist bicuculline (BIC, 50 μM). Scale bars: horizontal 50 ms, vertical 0.0003 I/I. C: simultaneous measurement of intracellular membrane potential by whole cell patch clamp electrode. DAMGO, CGP 55845, and coapplication of BIC and CGP 55845 increased the amplitudes of excitatory postsynaptic potentials (EPSPs) measured from an individual pyramidal cell somata. Scale bars: horizontal, 100 ms; vertical, 2 mV. D: histogram of relative changes in VSD excitatory signal amplitudes produced by DAMGO (DAMGO/Control) compared between anatomical layers for each stimulus in the train. Black, SO; blue, SP; yellow, SR; magenta, SLM. Stimulating electrodes were in SLM. E and F: same as D except stimulating electrodes were placed in SR (E) or SO (F).
EPSPs from the distal dendrites in SLM to the output layers in SP and SO (Fig. 1, A and B). The effect produced by MOR activation was similar in magnitude to the effect produced by GABAB receptor inhibition (CGP 55845, 10 μM). However, the effects produced by DAMGO and CGP 55845 were smaller than the effect produced by inhibiting both GABAB and GABA_A receptors (50 μM BIC, 10 μM CGP 55845). These effects were also observed using whole cell patch clamping in an individual pyramidal (Fig. 1C).

The effects of DAMGO on EPSPs evoked in SLM were normalized to control excitatory event amplitudes in all layers and for all stimuli in the train. In all layers of CA1, the effect of DAMGO became greater with each successive stimulus in the train (Fig. 1D, repeated-measures ANOVA, P < 0.0002 for all layers, n = 7, post hoc linear trend, P < 0.0001). The effectiveness of DAMGO was not the same in different layers of CA1 (Fig. 1E). When comparing the amplitude of the fifth stimulus in the train, the fractional change in excitatory event amplitude became significantly greater as it propagated away from its site of origin (repeated-measures ANOVA, P = 0.0018, post hoc Student–Newman–Keuls, P < 0.01 for SLM relative to SP and SO, P < 0.05 for SLM relative to SR, n = 7). Thus the activation of MORs in hippocampal CA1 increased EPSPs in SLM and facilitated their propagation to the output layers of CA1 pyramidal cells.

MOR activation had similar effects on EPSPs evoked in SR and SO (Fig. 1, E and F). When stimulating in SR, DAMGO increased EPSP amplitudes in all layers of CA1 and DAMGO was more effective on the EPSPs that occurred later in the train of stimuli (repeated-measures ANOVA, P < 0.0004, post hoc test for linear trend, P < 0.0001, n = 8). Similar observations were made on EPSPs evoked in SO (repeated-measures ANOVA, P < 0.04, post hoc test for linear trend, P < 0.003, n = 6). However, unlike observations made in SLM, when stimulating in SO or SR the effect of DAMGO on the amplitude of the fifth EPSP was not statistically different between anatomical layers of CA1.

Therefore despite the site of stimulation, activation of MORs increased EPSPs in all anatomical layers of hippocampal CA1. In a previous study we showed that the effect of MOR activation on a pair of EPSPs was the result of inhibition of the synaptic activation of GABA_A receptors (McQuiston and Saggau 2003). However, the effect of MOR activation on the synaptic activation of GABA_B receptors was not fully investigated. Therefore in an attempt to determine whether MOR activation also affects GABA_B-receptor–mediated synaptic inhibition, we examined the effect of MOR activation on EPSPs in the absence of GABA_A receptor function (in the presence of BIC, 50 μM).

MOR activation increases the amplitudes of excitatory activity driven by inputs in SLM in the absence of GABA_A receptor function

The distal dendrites of CA1 pyramidal cells receive two groups of excitatory afferents: the perforant path from the entorhinal cortex and excitatory inputs from the nucleus reuniens of the thalamus. To investigate the possibility that a GABA_B synaptic mechanism contributes to the MOR modulation of distal dendritic excitatory inputs in hippocampal CA1, we investigated the effect of DAMGO on SLM-evoked EPSPs in the presence of BIC (50 μM).

To examine the effect of MOR activation on the distal excitatory inputs to CA1, we stimulated the excitatory afferents in SLM (Fig. 2A, yellow arrow). This resulted in excitatory activity that propagated decrementally from SLM to SO (Fig. 2B, black traces). The amplitudes of these averaged signals (SLM, blue; SR, orange; SP, light blue; SO, red; Fig. 2A) were compared with each other for quantitation (Fig. 2, D–F). The five-pulse 20-Hz trained stimuli produced large excitatory events in SLM (Fig. 2B, bottom right). The later excitatory events were greatly reduced in size by a simultaneously occurring inhibitory event that occurred in SR, SP, and SO, but not SLM. Bath application of MOR agonist DAMGO (1 μM) increased the amplitude of the excitatory activity with the greatest change occurring on the last two excitatory events in SR, SP, and SO (Fig. 2B, blue traces). The effect of DAMGO was much smaller in SLM. Subsequent application of the MOR antagonist CTOP (1 μM) completely reversed the effect of DAMGO (Fig. 2B, orange traces) and application of the GABA_B receptor antagonist CGP 55845 (10 μM) produced a much greater increase in excitatory activity relative to the effects of DAMGO (Fig. 2B). A simultaneous fEPSP recording was performed in SLM (Fig. 2C). Neither DAMGO nor CGP 55845 had any effect on the slope of the fEPSP [two-tailed t-test, P = 0.56 (DAMGO), P = 0.86 (CGP 55845), n = 7].

The effects of DAMGO and CGP 55845 were normalized to control excitatory event amplitudes in all layers and for all stimuli in the train (Fig. 2D). In all layers of CA1, the effect of DAMGO became greater with each subsequent stimulus in the train and had little or no response on the first two stimuli (repeated-measures ANOVA, P < 0.0001 for all layers, n = 10, post hoc linear trend, P < 0.0001). Furthermore, the fractional change in amplitude during the fifth stimulus was significantly greater than the second stimulus in all anatomical layers (Bonferroni post hoc test, P < 0.001 in all layers, n = 10). The effectiveness of MOR agonist DAMGO was not the same in different layers of CA1 (Fig. 2E). When comparing the amplitude of the fifth stimuli in the train, the fractional change in excitatory event amplitude between anatomical layers was significantly different (repeated-measures ANOVA, P < 0.0018, n = 10). The fractional increase in excitatory event amplitude became significantly greater as it propagated away from its site of origin (post hoc Student–Newman–Keuls, P < 0.01 for SLM relative to SP and SO, P < 0.05 for SLM relative to SR, n = 10).

Because CGP 55845 appeared to produce a larger effect than did DAMGO on excitatory activity evoked in SLM (Fig. 2D), a comparison was made between the effect produced by CGP 55845 and the effect by DAMGO for each stimulus in the train and all layers of CA1 (Fig. 2F). Even though the effectiveness of DAMGO on excitatory event amplitude increased during subsequent stimuli in the train, the relative effect of DAMGO to CGP 55845 [(Fractional change by DAMGO/Fractional change by CGP 55845) × 100] decreased with each subsequent excitatory event in each anatomical layer (repeated-measures ANOVA, P < 0.0001, post hoc test for linear trend, P < 0.0001, for all anatomical layers, n = 10). Additional post hoc comparison showed that compared with CGP 55845, DAMGO was relatively more effective on the second excitatory event compared with the fifth (Bonferroni post hoc test,
MOR activation increases the amplitudes of excitatory inputs in SR in the absence of GABA_A receptor function

In addition to receiving excitatory inputs onto their distal dendrites in SLM, hippocampal CA1 pyramidal cells receive excitatory inputs from CA3 pyramidal cells onto their proximal apical dendrites in SR and their basal dendrites in SO. To investigate the possibility that a GABA_B synaptic mechanism contributes to the MOR modulation of CA1 SR excitatory inputs, we investigated the effect of DAMGO on SR-evoked EPSPs in the presence of BIC (50 μM).

We used similar procedures to those described in the previous section to examine the effect of MOR activation on excitatory activity driven by SC excitatory inputs in SR. A five-pulse 20-Hz train of stimuli was delivered with bipolar stimulating electrodes placed in SR to elicit large excitatory events in SR measured with VSD imaging (Fig. 3A, bottom left). The excitatory events decreased in amplitude as they propagated away from SR either out through SP and SO or back into SLM (Fig. 3A, black traces). Application of DAMGO (1 μM) increased the amplitude of the excitatory activity most clearly on the last three excitatory events in SO (Fig. 3A, blue traces) and the effect of DAMGO was completely reverse by the MOR antagonist CTOP (1 μM) (Fig. 3A, orange traces). Application of the GABA_B receptor antagonist (CGP 55845, 10 μM) produced a much greater increase in excitatory activity amplitude relative to the effects of DAMGO. This effect was largest on the last three excitatory events in the train.

The VSD signals in the presence of DAMGO and CGP 55845 were normalized to control VSD signals in all layers and for all stimuli in the train (Fig. 3, B and C). In all layers of CA1, the effect of DAMGO became larger on excitatory events occurring later in the stimulus train with little to no effect on the first two excitatory events (repeated-measures ANOVA, P < 0.0001 for all layers, n = 12, post hoc linear trend, P < 0.001 for all layers, n = 10). Furthermore, comparing the relative effect of DAMGO to CGP 55845 between anatomical layers showed DAMGO was relatively more effective at the site of stimulation (SLM) than the other layers in CA1 (repeated-measures ANOVA, P < 0.0001, Student–Newman–Keuls post hoc test, P < 0.001 for SLM relative to each other layer, n = 10).

\[ \frac{\text{DAMGO}}{\text{Control}} \] compared between anatomical layers for each stimulus in the train and each anatomical layer. Orange, CGP 55845/Control; blue, DAMGO/Control. Histogram of the change produced by DAMGO (DAMGO/Control) compared between anatomical layers for each stimulus in the train. Black, SO; blue, SP; yellow, SR; magenta, SLM. F: histogram of the change produced by DAMGO expressed as a percentage of the effect produced by CGP 55845 ([DAMGO/CGP 55845] × 100) compared between anatomical layers for each stimulus in the train. Black, SO; blue, SP; yellow, SR; magenta, SLM.
0.0001). Furthermore, the fractional change in excitatory event amplitudes was significantly greater on the fifth excitatory event compared with the second stimulus in all anatomical layers (Bonferroni post hoc test, \(P < 0.001\) in all layers, \(n = 12\)). Therefore when stimulating excitatory inputs in SR of CA1, DAMGO was more effective on the later excitatory events of the stimulus train in all layers of CA1.

When stimulating in SR, there was a significant difference in the effect of DAMGO on the last stimulus in the train when compared between anatomical layers in CA1 (Fig. 3C) (repeated-measures ANOVA, \(P < 0.0001\), \(n = 12\)). Post hoc analyses showed that the fractional increase in excitatory event amplitudes was larger in the output layers (SO and SP) compared with the dendritic layers SR and SLM (post hoc Student–Newman–Keuls, \(P < 0.001\) SLM relative to SP and SO, SR relative to SO; \(P < 0.01\) SR relative to SP, \(n = 12\)). In contrast, the effectiveness of DAMGO appeared to decrease in SLM when compared with SR, although this difference did not reach statistical significance. Thus when stimulating excitatory inputs in SR, DAMGO was most effective in the output layers of CA1.

Similar to the findings in SLM, the effect of DAMGO relative to CGP55845 on SR-evoked events decreased with each subsequent event in each anatomical layer (Fig. 3, B and D; repeated-measures ANOVA, \(P < 0.0001\), post hoc linear trend, \(P < 0.0001\), for all anatomical layers, \(n = 12\)). Further post hoc comparison showed that DAMGO was relatively more effective compared with CGP 55845 on the second excitatory event compared with the fifth event (Bonferroni post hoc test, \(P < 0.001\) for SO, SR, and SLM, \(P < 0.01\) for SR, \(n = 12\)). Moreover, DAMGO was more effective relative to CGP 55845 in SP and SR than the other layers in CA1 (repeated-measures ANOVA, \(P < 0.0001\), Student–Newman–Keuls post hoc test, \(P < 0.001\) SLM and SO relative to SP and SR, \(n = 12\)).

MOR activation increases the amplitudes of excitatory activity driven by inputs in SO in the absence of GABA_A receptor function

The SO of CA1 contains the basal dendrites of CA1 pyramidal cells that receive SC excitatory inputs from CA3 pyramidal cells and also contains the CA1 pyramidal cell efferents that project to the subiculum and deep layers of the entorhinal cortex. Thus stimulating in SO will activate both the inputs coming from CA3 as well as antidromically activate CA1 pyramidal cells. To investigate the possibility that a GABA_A synaptic mechanism contributes to MOR modulation of excitatory activity arising from SO, we investigated the effect of DAMGO on SO-evoked electrical activity in the presence of BIC (50 \(\mu M\)).

Using an identical procedure to the studies in SLM and SR, bipolar stimulating electrodes were placed in SO and a five-pulse 20-Hz train of stimuli was delivered to elicit excitatory events (Fig. 4A, top left). As observed when stimulating in either SR or SLM, the excitatory events decreased in amplitude as it propagated away from the site of origin (Fig. 4A, black traces). In this particular example, DAMGO (1 \(\mu M\)) increased the amplitude of the excitatory activity only modestly (Fig. 4A, blue traces). The effect of DAMGO was reversed by the MOR antagonist CTOP (1 \(\mu M\)) (Fig. 4A, orange traces) and application of CGP 55845 (10 \(\mu M\)) produced greater increases in the amplitude of excitatory events relative to the effects of DAMGO (Fig. 4A).

The amplitudes of excitatory events in the presence of DAMGO and CGP 55845 were normalized to control excitatory event amplitudes in all layers and for all stimuli in the train.
(Fig. 4, B and C). In all layers of CA1, the effect of DAMGO became greater on excitatory events occurring later in the train (repeated-measures ANOVA, linear trend, \( P < 0.0001, n = 12 \)). Additional post hoc analysis showed that the fractional change on the last excitatory event was significantly greater than the second stimulus in all anatomical layers (Bonferroni post hoc test, \( P < 0.001 \) in all layers, \( n = 12 \)). Therefore as was observed for the inputs in SR and SLM, when stimulating in SO of CA1, DAMGO was more effective in increasing the amplitudes of later excitatory events in all layers of CA1.

The fractional effect of DAMGO on the amplitudes of the fifth excitatory events (evoked in SO) was compared across the anatomical layers of CA1. However, unlike the inputs in SLM and SR, the fractional change in excitatory activity amplitude did not vary across layers of CA1 (Fig. 4C; repeated-measures ANOVA, \( P > 0.32, n = 12 \)). Thus DAMGO appeared to be equally effective in all layers of CA1 when excitatory activity was evoked in SO.

The effect of CGP 55845 on excitatory signal amplitudes appeared to be larger than DAMGO’s effect when the excitatory activity was evoked in SO (Fig. 4B). The percentage effect of DAMGO, relative to CGP 55845, decreased with each subsequent event in each anatomical layer (Fig. 4D; repeated-measures ANOVA, \( P < 0.0001 \), post hoc linear trend, \( P < 0.0001 \), for all anatomical layers, \( n = 12 \)). Additional post hoc comparison showed that DAMGO was relatively more effective on the second excitatory event compared with the fifth (Bonferroni post hoc test, \( P < 0.001 \) for SO, SR, and SLM, \( P < 0.01 \) for SR, \( n = 12 \)). Furthermore, comparing the effect of DAMGO on the last excitatory event between anatomical layers showed DAMGO was less effective in SLM than in the other layers in CA1 (repeated-measures ANOVA, \( P < 0.0001 \), Student–Newman–Keuls post hoc test, \( P < 0.001 \) SLM relative to all other layers, \( n = 12 \)).

**Suppression of GABA receptors occludes the effects of MOP receptor activation**

Because the effects of MOR activity in hippocampal CA1 were proposed to act through effects on inhibitory interneurons (Lupica and Dunwiddie 1991), the effects of MOR activation on excitatory activity in CA1 should be occluded in the absence of synaptic inhibition. This hypothesis was tested by blocking GABA\(_B\) receptors with CGP 55845 and then examining the effect of DAMGO in complete suppression of GABA receptor function. GABA\(_A\) receptors were blocked by bicuculline as in previous experiments.

When stimulating the afferents in SLM, DAMGO (orange) had no effect on the control excitatory events (black) observed in any layer of CA1 (Fig. 5A). A simultaneous whole cell patch-clamp recording from an individual pyramidal cell confirmed the voltage-sensitive dye measurements (Fig. 5A, right). These findings were observed consistently across slices and are summarized in Fig. 5B. Application of DAMGO had no effect on the amplitudes of the last excitatory events in any layer of CA1 when stimulating in SLM (paired \( t \)-test, control vs. DAMGO, \( P = 0.25 \) for all layers, \( n = 7 \)). Similar findings were observed when stimulating in SR (Fig. 5C; paired \( t \)-test, \( P = 0.72 \) for all layers, \( n = 6 \)) or stimulating in SO (Fig. 5D; paired \( t \)-test, \( P = 0.18 \) for all layers, \( n = 6 \)). Therefore regardless of stimulus site, DAMGO had no effect on the amplitudes of excitatory events in any layer of CA1 when GABA\(_B\) and GABA\(_A\) receptors were completely inhibited.

**MOP receptor activation inhibits GABA\(_B\) IPSPs in all layers of CA1**

Because MOP receptor activation affected excitatory activity in all layers of CA1 and this effect was occluded by blockade of GABA\(_B\) receptors, the effect of MOP receptor...
activity on GABA_B IPSPs was investigated in all layers of CA1. GABA_B IPSPs were evoked by electrically stimulating slices at the border of SLM and SR, or at the border of SO and SP, where there are large concentrations of inhibitory interneurons and their axons. GABA_B IPSPs were isolated by blocking excitatory synaptic transmission with the glutamatergic ionotropic receptor inhibitors DNQX (30 μM) and APV (50 μM) in the presence of bicuculline (50 μM). In some experiments, the concentration of extracellular calcium was increased to 2.5 mM and the experiments were performed at room temperature. This increased the size of the VSD signals arising from the activation of GABA_B IPSPs. Under these conditions, stimulation at the border of SLM/SR produced large hyperpolarizing signals (Fig. 6A, black traces) in all layers of CA1. The GABA_B IPSPs were inhibited by DAMGO (Fig. 6A, blue traces) and the effect of DAMGO was reversed by the MOP

**FIG. 5.** Activation of μ-opioid receptors in the absence of GABA receptor function (in the presence of CGP 55845 10 μM, BIC 50 μM) has no effect on excitatory activity in CA1. A: control VSD responses to a train (5 × 20 Hz) of electrical stimuli in SLM produced excitatory activity in each anatomical layer of CA1. VSD signals: Control, black; DAMGO, orange. A simultaneous whole cell patch-clamp recording (right) was also performed. Control, black; DAMGO, orange. Horizontal scale bars: 50 ms; vertical scale bars: 0.0002 ΔI/I and 2 mV (whole cell). B: histogram of the change in excitation signal amplitude produced by DAMGO (1 μM) relative to control excitatory VSD signal amplitudes generated in the absence (black) and presence of GABA_B receptor blockade (magenta, CGP 55845 10 μM, with BIC 50 μM) after electrically stimulating in SLM. C and D: as in B, except excitatory activity was generated in SR (C) and SO (D).

**FIG. 6.** DAMGO inhibits GABA_B inhibitory postsynaptic potentials (IPSPs) in all anatomical layers of hippocampal CA1. A: VSD measurements showing isolated GABA_B IPSPs [BIC 50 μM, 6,7-dinitroquinoxaline-2,3-dione (DNQX) 30 μM, and D-2-amino-5-phosphonovaleric acid (APV) 50 μM] in all anatomical layers of CA1 evoked by stimulation (5 pulses, 20 Hz) at the border of SLM and SR. Right: simultaneous whole cell recording from an individual pyramidal cell. Traces: Control, black; DAMGO, blue; CTOP, orange; CGP 55845, green. Horizontal scale bars: 50 ms; vertical scale bars: 0.0002 ΔI/I and 2 mV (whole cell). B: histogram of percentage inhibition of GABA_B IPSP by DAMGO relative to complete inhibition by CGP 55845 [(DAMGO/CGP 55845) × 100] for each anatomical layer after stimulation at the border of SR and SLM. C: as in B, except stimulation occurred at the border of SP and SO.
receptor antagonist CTOP (Fig. 6A, orange traces). The GABA_B receptor antagonist CGP 55845 completely blocked the hyperpolarizing signals in all layers of CA1 (Fig. 5A, green traces). These data were confirmed by simultaneously measuring the IPSP in a pyramidal neuron by whole cell patch clamping (Fig. 5A, right). The DAMGO-induced fractional change in GABA_B IPSP amplitudes evoked by stimulating in SLM/SR was not different between layers of CA1 (Fig. 5B; repeated-measures ANOVA, $P = 0.18$, $n = 13$). Similarly, the effect of DAMGO on GABA_B IPSPs evoked at the border of SO/SP was not significantly different across all layers of CA1 (Fig. 6C; repeated-measures ANOVA, $P = 0.41$, $n = 12$). Thus DAMGO appeared to equivalently decrease GABA_B IPSP amplitudes in all layers of CA1.

**DISCUSSION**

These VSD studies have shown that MOR activation decreased GABA_B IPSPs in all layers of hippocampal CA1. MOR inhibition of GABA_B synaptic activity in CA1 resulted in an increase in excitatory activity regardless of the excitatory afferents producing the response. The increase in excitatory activity was either absent or very small on the first two excitatory events in a train of stimuli. The last stimulus in the train showed the maximal effect of MOR activation. Interestingly, although the last stimulus of a train showed the maximal effect of MOR activation, MOR activation was less efficacious relative to complete inhibition of GABA_B synaptic function by CGP 55845. Nevertheless, MOR activation inhibits synaptic activation of GABA_B receptors, resulting in reduced GABA_B synaptic hyperpolarizations in all layers of CA1. This MOR inhibition resulted in an increase in excitatory synaptic events in all layers of hippocampal CA1, but the relative effectiveness across layers differed depending on the specific excitatory afferents stimulated.

Previous work showed that MOR activation facilitated excitatory synaptic input in SLM, SR, and SO in hippocampal CA1 (McQuiston and Saggau 2003). These studies showed that MOR activation could increase the amplitude of a pair of excitatory synaptic events by inhibiting the synaptic activation of GABA_A receptors. This effect did not appear to involve a modulation of GABA_B synaptic function. However, these studies examined only a pair of excitatory synaptic events (50-ms interval) and measured signals only 250 ms after the first synaptic event. The present studies measured $\leq 1,700$ ms after the first synaptic event and used a train of five stimuli (50-ms intervals) to examine a potential inhibition of GABA_B synaptic function (Lupica et al. 1992). Although MOR activation had little to no effect on the first two excitatory events (McQuiston and Saggau 2003), there was a significant increase in the excitatory events at the end of the stimulus train. This may be attributable to the slow responses produced by metabotropic GABA_B receptors and/or to a possible requirement for multiple stimuli to release GABA from a subset of interneurons that activate GABA_B receptors (Gupta et al. 2000; Markram et al. 1998; Reyes et al. 1998; Thomson 1997). Thus by taking longer measurements and using multiple stimuli, this study was able to show that MOR activation can modulate the synaptic activation of GABA_B receptors in hippocampal CA1, data not previously observed (McQuiston and Saggau 2003).

MOR suppression of GABA_B synaptic function had different effects in different layers. When stimulating excitatory inputs in SLM, MOR suppression of GABA_B synaptic function had a much greater effect as the excitatory activity propagated toward SP and SO. Similarly, the effect on excitatory activity arising in SR was largest in SP and SO but smaller or the same size in SLM. When stimulating in SO, the effect of MOR activation was not different between the different layers of CA1. If there was a significant MOR suppression of GABA_B synaptic function in SR and SLM after stimulation in SO, it would be expected that the excitatory events would increase further as they propagated into SR and SLM. However, this effect was not observed. Rather, the data suggest that MOR activation had little effect on GABA_B synaptic function in SR and SLM after stimulating in SO. Therefore the greatest effect of MOR suppression of GABA_B synaptic function was in the output layers (SP and SO) of the hippocampal CA1. This is consistent with previous anatomical studies showing that MORs are concentrated in SP (Arvidsson et al. 1995; Atweh and Kuhar 1977; Crain et al. 1986; Ding et al. 1996; Herkenham and Pert 1980; Mansour et al. 1987, 1994, 1995; McLean et al. 1987) and with physiological data that showed that MOR activation hyperpolarized interneurons innervating SP more frequently than interneurons innervating the dendritic layers of CA1 pyramidal cells (Svoboda et al. 1999). However, previous anatomical studies described moderate concentrations of MORs in SLM (Arvidsson et al. 1995; Atweh and Kuhar 1977; Crain et al. 1986; Ding et al. 1996; Herkenham and Pert 1980; Mansour et al. 1987, 1994, 1995; McLean et al. 1987) and showed that interneurons projecting to SLM frequently express MORs (Bausch et al. 1995; Drake and Milner 1999, 2002; Kalyuzhny and Wessendorf 1997). This is somewhat inconsistent with the relatively small effect of MOR activation observed in SLM in this VSD study. One potential explanation for this apparent discrepancy is that the stimulation protocols in this VSD study were not sufficient to release enough GABA from the interneurons that activate GABA_A receptors in SLM. Another possible explanation is that MOR activation blocks GABA release onto GABA_A receptors (McQuiston and Saggau 2003) but not onto GABA_B receptors in SLM. This latter explanation seems plausible because when stimulating in SO or SR, the effect of MOR activation relative to complete GABA_B receptor inhibition was much smaller in SLM compared with that in the other layers. When stimulating in SLM, both MOR activation or GABA_B receptor inhibition have almost no effect in SLM. Therefore we propose that MOR activation has a smaller effect on the synaptic activation of GABA_B receptors in SLM and its greatest effect is in the output layers (SP and SO) of CA1.

Even though MOR activation had little to no impact on excitatory inputs to SLM, the propagation of excitatory activity out of SLM was facilitated by MOR suppression of GABA_B synaptic activity in SR, SP, and SO. Interestingly, it was previously hypothesized that the primary function of the excitatory inputs in SLM is to suppress activity in SR and SP by a GABA_B synaptic process (Dvorak-Carbone and Schuman 1999). This inhibition produced by excitatory inputs in SLM was proposed to modulate the induction of synaptic plasticity in the SC pathway (Remondes and Schuman 2002). Therefore MOR suppression of GABA_B synaptic function in SR and SP may facilitate SC-driven firing of action potentials in pyram-
nal cells and favor the expression of long-term potentiation over long-term depression during SLM modulation of SC synaptic plasticity (Remondes and Schuman 2002).

Activation of excitatory input to the distal dendrites of CA1 pyramidal cells in SLM normally produces a net inhibitory effect in the soma of CA1 pyramidal cells (Ang et al. 2005; Dvorak-Carbone and Schuman 1999) and not an excitation because of their distance from the soma (Jarsky et al. 2005). However, during theta rhythms, excitatory inputs in SR and excitatory inputs in SLM release transmitter rhythmically but completely out of phase with each other. This results in the activation of excitatory inputs in SLM and SR being separated in time by about 50 to 100 ms (Buzsáki 2002). Interestingly, stimulation in SLM can produce excitatory events and action potentials in CA1 pyramidal cells if the stimulation in SLM is preceded by stimulation of SC inputs in SR (Ang et al. 2005; Jarsky et al. 2005). Therefore facilitation of SR inputs by MOR activation might favor excitation of CA1 pyramidal cells by coordinated activation of inputs in SLM and SR (Jarsky et al. 2005) as occurs during theta oscillations during attentive behavior (Ang et al. 2005; Buzsáki 2002).

Perhaps the most significant effect of MOR-induced suppression of GABA$_B$ synaptic activity was in the output layers of CA1. Despite the layer of stimulation, the effect of MOR activation was always maximal in SP. Therefore all inputs will be more effective at driving CA1 pyramidal cells to threshold and thus significantly alter the coding of information in CA1 pyramidal cells (Faulkner et al. 1998, 1999; Whittington et al. 1998). Furthermore, all inputs will have a lower threshold to evoke somatic and back-propagating action potentials. This increased excitability and increased probability in producing back-propagating action potentials will change the ability of individual inputs to induce long-term synaptic changes (Magee and Johnston 1997). Thus it is expected that the effects of MOR at the soma of pyramidal cells, regardless of the source of excitatory synaptic input, will have significant effects on coding of information in CA1 as well as changing the ability of different inputs to produce changes in synaptic efficacy.

In summary, MOR suppression of GABA$_B$ synaptic function in hippocampal CA1 influences all inputs and the output of CA1 pyramidal cells. The result of the modulation of GABA$_B$ synaptic function by MOR activation is likely to be complex. The activation of MOR will likely alter coding of electrical activity, change the network requirements to induce synaptic plasticity, and modify the integration between different input pathways in an individual pyramidal cell. It will be of interest to determine how each of the effects of MOR activation is affected by chronic MOR activation and how this influences hippocampal CA1 network function in both the presence and the absence of acute MOR activation.

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