Orphanin FQ/Nociceptin Inhibits Synaptic Transmission and Long-Term Potentiation in Rat Dentate Gyrus Through Postsynaptic Mechanisms

TZU-PING YU AND CUI-WEI XIE
Department of Psychiatry and Biobehavioral Sciences, Neuropsychiatric Institute, University of California, Los Angeles, California 90024-1739

Yu, Tzu-Ping and Cui-Wei Xie. Orphanin FQ/nociceptin inhibits synaptic transmission and long-term potentiation in the rat dentate gyrus through postsynaptic mechanisms. J. Neurophysiol. 80: 1277–1284, 1998. Orphanin FQ/nociceptin (OFQ), a recently characterized natural ligand for the opioid receptor-like 1 (ORL₁) receptor, shares structural similarity to the endogenous opioids. Our previous study found that OFQ, like classical opioids, modulated synaptic transmission and long-term potentiation (LTP) in the hippocampal CA1 region, suggesting a modulatory role for OFQ in synaptic plasticity involved in learning and memory. In the present study we investigated the action of OFQ in the dentate gyrus and explored possible underlying cellular mechanisms. Field potential recordings showed that OFQ significantly inhibited excitatory synaptic transmission and LTP induction in the dentate lateral perforant path. In the presence of OFQ, the excitatory postsynaptic potential (EPSP) slope-population spike (E-S) curve was shifted to the right, and no significant change was found in paired-pulse facilitation, suggesting a postsynaptic mechanism responsible for the inhibition of synaptic transmission. Under whole cell voltage-clamp conditions, bath application of OFQ activated K⁺ currents in most granule cells tested at a holding potential of −50 mV, suggesting that OFQ could reduce the excitability of dentate granule cells by hyperpolarizing cell membranes. OFQ also inhibited the amplitude of N-methyl-D-aspartate (NMDA) receptor–mediated excitatory postsynaptic currents (EPSCs) without affecting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated EPSCs. This inhibition was not blocked by opioid receptor antagonists. Furthermore, the inward currents evoked by focal application of NMDA to granule cells were suppressed by OFQ in a dose-dependent manner, suggesting that OFQ may suppress LTP by inhibiting the function of postsynaptic NMDA receptors. These results demonstrate that OFQ may negatively modulate synaptic transmission and plasticity in the dentate gyrus through postsynaptic mechanisms, including hyperpolarization of granule cells as well as inhibition of the function of postsynaptic NMDA receptors/channels in dentate granule cells.

INTRODUCTION

It is known that classical opioids have receptor-specific actions on synaptic transmission and long-term potentiation (LTP) in the hippocampus, which linked the opioids to the modulations of certain forms of learning and memory (for review see Simmons and Chavkin 1996). The recently isolated heptadecapeptide orphanin FQ/nociceptin (OFQ), an endogenous ligand for opioid-receptor-like 1 receptors (ORL₁), shares sequence similarities with opioid peptides, particularly with dynorphin A (Meunier et al. 1995; Reinscheid et al. 1995). Because of its structural similarity to classical opioids and the high-density of ORL₁ receptors in the rat hippocampus (Anton et al. 1996), it is of interest to explore whether OFQ, like opioids, modulates hippocampal synaptic function. Similar to dynorphin A, OFQ was reported to function as an inhibitory modulator for synaptic function in the hippocampus. This peptide is capable of inhibiting calcium (Ca²⁺) currents through voltage-gated Ca²⁺ channels in dissociated hippocampal neurons (Knofflach et al. 1996), inducing hyperpolarizing currents via inward-rectifying K⁺ channels in hippocampal CA3 cells (Ikeda et al. 1997) or suppressing synaptic transmission in the CA1 region of hippocampal slices (Yu et al. 1997).

Our previous study reported an inhibitory effect of OFQ on LTP in the Schaffer collateral-CA1 pathway, indicating that activation of the ORL₁ receptor by OFQ may modulate hippocampal synaptic plasticity involved in learning and memory (Yu et al. 1997). This idea was supported by a recent finding of impaired spatial learning after the intrahippocampal injection of OFQ (Sandin et al. 1997). Nevertheless, the cellular mechanisms underlying the inhibitory actions of OFQ on LTP remain to be addressed. In addition to the CA1 region, a high level of ORL₁ receptor expression was also found in the hippocampal dentate gyrus, particularly in the molecular layer of the dentate (Anton et al. 1996; Mollereau et al. 1994), where perforant path terminals and granule cell dendrites are located. Little is known regarding the actions of OFQ in this region. This study examined the action of OFQ in the dentate gyrus, demonstrating a strong inhibitory effect on both synaptic transmission and LTP induction at perforant path-granule cell synapses. The possible cellular mechanisms for its effects were further investigated.

METHODS

Slice preparation

Transverse hippocampal slices (500 and 300 μm for extracellular and whole cell recordings, respectively) were prepared from male Sprague-Dawley rats (24 to 40 days old) and maintained in a holding chamber with oxygenated artificial cerebrospinal fluid (ACSF) at 32°C. The ACSF contained (in mM) 120 NaCl, 25 NaHCO₃, 3.3 KCl, 1.23 NaH₂PO₄, 1.8 CaCl₂, 1.2 MgCl₂, and 10 d-
glucose. In experiments recording \textit{N}-methyl-\textit{d}-aspartate (NMDA) currents, Mg\textsuperscript{2+} was omitted from the ACSF.

**Extracellular field potential recordings**

After 1-h incubation in the holding chamber, the slice was transferred to a submersion recording chamber held at 32°C. Extracellular field potentials were evoked by constant stimulus pulses (0.1 ms, 0.06–0.4 mA) delivered by a stimulator (Grass model S88) through sharpened monopolar tungsten electrodes and recorded with glass micropipettes filled with 2 M NaCl. In the CA1 region, population spikes and excitatory postsynaptic potentials (EPSPs) were recorded from the pyramidal cell layer and the dendritic layer (stratum radiatum), respectively, in response to stimulation of the Schaffer collaterals. In the dentate gyrus, the lateral perforant path was stimulated in the outer molecular layer, and the evoked responses were recorded from the granule cell and dendritic layers.

Paired-pulse facilitation was measured by application of paired-pulse stimuli to presynaptic fibers. The stimulus intensity that only induced EPSPs without triggering population spikes (0.05–0.1 mA, 0.1 ms in duration with an interstimulus interval of 50 ms) was chosen. In the CA1 region where EPSPs were reduced by OFQ, the stimulus intensity was adjusted so that the amplitude of the response to the first pulse matched the amplitude of the baseline response. The amount of paired-pulse facilitation was expressed as the percent increase in the amplitude of the second response relative to the first one, which was taken as 100%.

In LTP experiments, test pulses that evoked 50% of the maximum response were delivered at 0.017 Hz to collect baseline responses in ACSF. OFQ was then bath applied to the slice for 10 or 20 min as indicated, during which responses evoked by test pulses were recorded. At the end of OFQ perfusion, a high-frequency stimulation (1-s train of pulses at 100 Hz) was applied to the lateral perforant path to induce LTP in the presence of OFQ. The peptide was washed off immediately after the train. Post-train responses evoked by the same test pulses for baseline collection were recorded during 30-min washing. Taking the baseline response as 100%, the amount of LTP was expressed as a percentage of change in the population spike amplitude or EPSP slope 30 min after the train. An increase of \(\geq 20\%\) from the baseline was considered as significant LTP, which was observed in \(>80\%\) of the slices examined. When the effect of OFQ was tested, LTP was examined from a pair of slices (1 for control, 1 for OFQ treatment) obtained from the same rat. If significant LTP was not observed in the control slice, the data obtained from the OFQ-treated slice of the same rat were excluded.

The recorded responses were amplified, digitized, and stored on computer disks for off-line analysis. Data were presented as means \(\pm\) SE. Paired-pulse facilitation as well as membrane potentials of dentate granule cells in the absence and presence of OFQ were compared by using paired Student’s \(t\)-test. Tetanus-induced LTP in the control and OFQ-treated groups were compared by using unpaired Student’s \(t\)-test, provided that the measures were obtained separately from two groups of slices. Difference was considered significant at the level of \(P < 0.05\), \(P < 0.01\), or \(P < 0.001\) as indicated.

**Whole cell voltage-clamp recording**

The patch pipettes were pulled with the use of a Flaming Brown micropipette puller (model P-87). Unless otherwise indicated, the

![FIG. 1. Orphanin FQ/nociceptin (OFQ) inhibits population spikes but has no effect on excitatory postsynaptic potential (EPSP) slope in the dentate gyrus. A: time course of synaptic depression after the perfusion of OFQ. Population spikes (top panel) and EPSPs (middle panel) were representative recordings obtained at different time (as indicated in the plot). Bottom panel (a): responses evoked in artificial cerebrospinal fluid (ACSF) were collected as baseline responses; (b) bath application of 10 \(\mu\)M OFQ (solid bar) caused a marked reduction in the amplitude of the population spike but did not affect the EPSP slope; (c) inhibitory effect on the population spike could be washed out completely within 20 min. Calibration bars: 2.5 mV, 10 ms. B: histogram summarizes the effect of OFQ on the amplitude of population spikes and EPSP slope. Inhibitory effect of OFQ was determined 10 min after the start of perfusion. In the presence of 1 \(\mu\)M OFQ, population spikes were significantly reduced compared with the controls (* \(P < 0.001\)). Slope of EPSP was not affected by OFQ. C: inhibitory effect of OFQ on population spikes was concentration dependent. Bath application of OFQ at 0.25-, 1-, and 5-\(\mu\)M concentrations reduced the peak amplitude of the population spike by 17 \(\pm\) 2\% (\(n = 4\)), 24 \(\pm\) 2\% (\(n = 6\)), and 34 \(\pm\) 3\% (\(n = 4\)), respectively.]
internal solution of the recording pipette contained (in mM) 110 methyl potassium sulfate (KMeSO₄), 10 KCl, 10 Na-[2-hydroxyethyl]piperazine-Ν°-[2-ethanesulfonic acid] (HEPES), 2 MgCl₂, 10 ethylene glycol-bis(β-aminoethyl ether)Ν°Ν°-tetraacetic acid (EGTA), 1 CaCl₂, and 2 Mg-ATP at pH 7.2. In some experiments, KMeSO₄ and KCl were replaced by CsCl (110 mM) and tetraethylammonium chloride (20 mM), respectively. Individual granule cells in the slice were visualized with a Zeiss AxioScope fitted with a ×40 water immersion objective and differential interference contrast optics in conjunction with infrared video microscopy with an overall magnification of ×400. Cells in the granule cell body layer, characterized by small (~10–μm) diam and granular cell bodies, were selected. Liquid junction potentials were corrected before seals were formed. Cells were usually held at ~70 mV with a patch-clamp amplifier (Axopatch-200A, Axon Instruments). The stimulus intensity was adjusted to elicit synaptic currents with an amplitude <200 pA. Measured series resistance was ~15–20 MΩ and could be compensated up to 80%.

To determine the effect of OFQ on membrane currents of granule cells, the cells were constantly voltage clamped at ~70 mV, and ramp-pulse voltage commands (0.01 Hz, from ~50 to ~110 mV) were used to monitor the I-V relationship before, during, and after perfusion of OFQ. Excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation of the lateral perforant path and the inward currents induced by pressure ejection of NMDA were recorded from dentate granule cells. For stimulation-evoked EPSCs, the stimulus intensity was in the range of 0.01–0.08 mA, with a duration of 0.1 ms. For NMDA-induced inward currents, a micropipette filled with 2 mM NMDA was positioned with a duration of 0.1 ms. For NMDA-induced outward currents, a micropipette filled with 2 mM NMDA and the stimulus intensity was adjusted to elicit synaptic currents with an amplitude <200 pA. Measured series resistance was ~15–20 MΩ and could be compensated up to 80%.

To determine the effect of OFQ on membrane currents of granule cells, the cells were constantly voltage clamped at ~70 mV, and ramp-pulse voltage commands (0.01 Hz, from ~50 to ~110 mV) were used to monitor the I-V relationship before, during, and after perfusion of OFQ. Excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation of the lateral perforant path and the inward currents induced by pressure ejection of NMDA were recorded from dentate granule cells. For stimulation-evoked EPSCs, the stimulus intensity was in the range of 0.01–0.08 mA, with a duration of 0.1 ms. For NMDA-induced inward currents, a micropipette filled with 2 mM NMDA was positioned ~50 μm from the patched neuron, and a brief pressure ejection (10 ms, 15 psi) of NMDA was applied with the use of a Picospritzer (General Valves). For all the experiments, the membrane potentials of granule cells were transiently changed from ~70 mV to different voltages with the use of voltage steps applied 200 ms before synaptic stimulation or 1 s before pressure ejection of NMDA. The input resistance of the cell was monitored by examining the deflection of steady-state holding currents during the voltage steps. The cells examined in all the experiments had an input resistance ranging from 150 to 600 MΩ. Recorded currents were filtered at 2 kHz and analyzed with the use of pClamp 6 (Axon Instruments).

**Drug application**

OFQ and des-Phe¹-OFP were synthesized on 2-chlorotrityl resin (Anaspec) with standard Fmoc procedures, which was described previously (Yu et al. 1997). Both peptides were dissolved in deionized water and prepared as 5-mM stock solutions stored at ~20°C. NMDA and dimethylsulfoxide (DMSO) were obtained from Sigma. Other drugs were purchased from RBI. All drugs, except NMDA, were bath applied. To isolate NMDA receptor-mediated synaptic responses, 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) dissolved in DMSO and 50 μM (−)-bicuculline methiodide (BMI) were included in the ACSF to eliminate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and γ-aminobutyric acid-A (GABA_A) receptor-mediated components. AMPA receptor-mediated EPSCs were recorded in the medium containing 50 μM BMI and 40 μM (−)-2-amino-5-phosphono-20 min of perfusion of drug-free ACSF. In some experiments, 1 μM tetrodotoxin (TTX) was included in the ACSF to eliminate action potentials, and NMDA (2 mM) was focally applied to the granule cell after the whole cell configuration was obtained.

**RESULTS**

**Effect of OFQ on synaptic transmission in the dentate gyrus**

After bath application of OFQ, the effect of the peptide on dentate synaptic transmission was examined by recording population spikes and EPSPs in response to stimulation of the lateral perforant path. A marked reduction in the amplitude of the population spike was observed ~10 min of perfusion of OFQ (10 μM) perfusion (Fig. 1A). This inhibitory effect could be reversed ~20 min of perfusion of drug-free ACSF. In contrast to the significant inhibition of population spike (75 ± 2%, means ± SE, n = 17, P < 0.01), we observed

![FIG. 2. Different mechanisms underlie the effects of OFQ in the CA1 and dentate gyrus. A: in CA1, OFQ may inhibit synaptic transmission through a postsynaptic mechanism. A1: at a given presynaptic fiber volley, EPSP slope was reduced in the presence of OFQ (1 μM). A2: relationship of EPSP slope and population spikes was not affected by OFQ. A3: OFQ significantly increased paired-pulse facilitation (n = 7; * P < 0.05). B: OFQ inhibits synaptic transmission in the dentate gyrus through a postsynaptic mechanism. B1: OFQ (1 μM) did not change the relationship of presynaptic fiber volley and EPSP slope. B2: OFQ shifted the EPSP slope-population spike curve to the right. B3: paired-pulse facilitation was not affected by OFQ (n = 7).](http://jn.physiology.org/ by 10.22033.6 on October 28, 2016)
Our results showed that neither naloxone (10 μM; data not shown) nor naltrexone and the selective κ receptor antagonist nor-BNI (1 μM) blocked the inhibition caused by OFQ (1 μM; data not shown).

**Different mechanisms for OFQ-induced inhibition in dentate gyrus and CA1**

To investigate the possible mechanisms underlying the inhibitory action of OFQ, we compared its effects in the CA1 and dentate gyrus in several tests. First, the slope of field EPSP was plotted versus the amplitude of the presynaptic fiber volley. As shown in Fig. 2A, the slope of EPSP evoked at a given presynaptic fiber volley was reduced by OFQ in the CA1 region, demonstrating an OFQ-induced reduction of synaptic strength. Second, we plotted the amplitude of population spikes versus EPSP slopes obtained at different stimulus intensities. OFQ caused no evident shift of the EPSP slope-population spike (E-S) curve in the CA1 (Fig. 2A), suggesting no significant change in the excitability of postsynaptic neurons. Finally, we investigated the effect of OFQ on paired-pulse facilitation, which is known as an index of presynaptic modification (Schulz et al. 1994; Zucker 1989). We found that the inhibition of synaptic transmission by OFQ in the CA1 region was associated with a significant increase in paired-pulse facilitation (11 ± 4%, P < 0.05, Fig. 2A). In contrast to what we found in the CA1 region, in the dentate gyrus the relationship of presynaptic fiber volley with EPSP slope was not affected by OFQ (Fig. 2B), and no significant change in paired-pulse facilitation was observed in the presence of OFQ (Fig. 2B). Instead, OFQ caused a rightward shift of the E-S curve, suggesting that OFQ attenuated the firing probability of the postsynaptic neuron in the dentate gyrus (Fig. 2B).

**OFQ-induced potassium current in dentate granule cells**

One possible mechanism underlying the inhibitory effect of OFQ on dentate synaptic transmission is OFQ-induced hyperpolarization of dentate granule cells. Previous studies reported that OFQ could hyperpolarize neurons through activation of K+ currents in various brain regions (Connor et al. 1996; Ikeda et al. 1997; Vaughan and Christie 1996; Vaughan et al. 1997). We therefore examined whether OFQ could hyperpolarize dentate granule cells and induce a K+ current. Our results showed that OFQ (1 μM) hyperpolarized membrane potentials of dentate granule cells by −12 ± 1 mV (n = 4, P < 0.01, paired t-test). At a holding potential of −50 mV, bath application of OFQ (1 μM) evoked an evident outward current in 11 of 15 granule cells (98 ± 18 pA, n = 11). This outward current was accompanied by a reduction of input resistance (by 30 ± 4%, n = 7) and could be completely washed out in 30 min (Fig. 3A). Four of 15 granule cells showed small outward currents (<20 pA) in response to OFQ. Data from these four cells were not included because these small currents could not be reversed by 30-min washing; it was therefore not distinguishable from baseline fluctuations.

The I-V relationships of the cell before and during perfusion of OFQ were monitored by application of ramp-pulse voltage steps to the cell (Fig. 3B). OFQ-induced outward current appeared to be a K+ current because it reversed at −85 ± 2 mV (n = 6), which was close to the predicted K+ equilibrium potential by Nernst equation (−93 mV), given 120 and 3.3 mM for intra- and extracellular K+, respectively. When the extracellular K+ concentration of the perfusion...
medium was 8 mM, the reversal potential of the current shifted, as expected from the Nernst equation (Fig. 3C).

**Inhibitory effect of OFQ on LTP**

Because synaptic transmission in the dentate gyrus was strongly modulated by OFQ, we examined whether OFQ could also affect LTP in the dentate gyrus. We found that, although OFQ (1 μM) did not cause any significant reduction in the slope of field EPSPs evoked by low-frequency test pulses, the peptide strongly inhibited the induction of LTP by high-frequency stimulation applied to the lateral perforant path (Fig. 4A). LTP was significantly smaller in both population spikes (114 ± 4%, n = 9) and EPSP slope (107 ± 6%, n = 7) compared with the controls (149 ± 7%, n = 9, P < 0.001 and 144 ± 2%, n = 7, P < 0.001, respectively, Fig. 4B). This OFQ-induced inhibition was not reversed by nor-BNI (1 μM, Fig. 4C), suggesting that the effect of the peptide was not mediated through κ receptors.

**Effects of OFQ on NMDA receptor–mediated currents**

The induction of LTP at the lateral perforant path-dentate granule cell synapse is known to be NMDA receptor dependent (Colino and Malenka 1993; Hanse and Gustafsson 1992). It is likely that OFQ may depress the induction of LTP by inhibiting the function of NMDA receptors/channels on granule cells. To investigate this possibility, we examined the effect of OFQ on NMDA receptor–mediated EPSCs (NMDA EPSCs). NMDA EPSCs evoked by stimulation of the lateral perforant path were recorded from dentate granule cells in Mg2+-free ACSF containing 50 μM BMI and 20 μM DNQX. As shown in Fig. 5A, OFQ attenuated NMDA EPSCs recorded with either K+ or Cs+-filled micropipettes, whereas EPSCs mediated by AMPA receptors were not affected. The reduction of NMDA EPSCs in the presence of 0.3, 0.5, and 1 μM OFQ was 18 ± 1% (n = 5), 30 ± 3% (n = 7), and 37 ± 3% (n = 16), respectively, with the use of K+-filled recording pipettes (Fig. 5B). NMDA EPSCs recorded with Cs+-filled pipettes were inhibited by OFQ (1 μM) to a similar degree (44 ± 7%, n = 4). The inhibitory effect of OFQ was not reversed by naloxone or nor-BNI (Fig. 5B), suggesting that the action of the peptide was not mediated through activation of opioid receptors. In addition, the inactive analog of OFQ, des-Phe1-OFQ (1 μM), as reported previously (Yu et al. 1997), did not have any significant effect on NMDA EPSCs (Fig. 5C).

Because the inhibition of NMDA EPSCs could result from either suppression of postsynaptic NMDA receptor function or reduced transmitter release from presynaptic terminals, we directly applied NMDA (2 mM) to the postsynaptic neuron and examined whether OFQ caused any change in NMDA-evoked inward currents. The inward currents were

![FIG. 4. OFQ inhibits long-term potentiation (LTP) in the dentate lateral perforant path. A: LTP induced by high-frequency stimulation (HFS, 1) was blocked by 1 μM OFQ. The stimulus intensity that caused 50% of the maximum response was used to collect the baseline. HFS was then delivered to the control slice in drug-free ACSF. For the OFQ-treated slice, the peptide was bath applied to the slice for 10 min. HFS was applied in the presence of OFQ. Immediately after the tetanus, the peptide was washed off. Responses were continuously recorded for 30 min. As shown here, the control slice displayed a 53% increase in EPSP slope relative to the baseline, whereas the OFQ-treated slice failed to show LTP. B: histogram summarizes the depressant effect of OFQ (1 μM) on LTP in the dentate gyrus. All the experiments were performed with the same paradigm described in A. HFS-induced potentiation of both population spike amplitude and EPSP slope were determined 30 min after HFS, relative to the pretrain baseline response. Compared with control slices, LTP of both population spikes and EPSP slope were significantly suppressed in OFQ-treated slices (*P < 0.001). C: κ receptor antagonist nor-Binaltorphimine (nor-BNI) did not reverse the inhibitory effect of OFQ on dentate LTP. LTP of both population spikes and EPSP slope in OFQ-treated slices (1 μM, 20 min) were significantly inhibited compared with the control groups (ACSF; *P < 0.01). Nor-BNI (1 μM) alone did not significantly affect LTP. Addition of nor-BNI (1 μM) into the perfusion medium 10 min after starting OFQ (1 μM) application did not reverse significant inhibition of LTP by OFQ (*P < 0.01).]
evoked by focal application of NMDA to the dentate granule cells in the Mg$^{2+}$-free ACSF containing 50 μM BMI and 1 μM TTX (Fig. 6A). During bath application of OFQ, the evoked inward currents were dramatically reduced, suggesting that OFQ directly acted on postsynaptic neurons to inhibit NMDA EPSCs. OFQ at 0.5, 1, and 5 μM reduced the current by 23 ± 4% (n = 6), 34 ± 2% (n = 11), and 57 ± 4% (n = 5) of the baseline, respectively (Fig. 6B).

**DISCUSSION**

This study demonstrates that OFQ is able to suppress synaptic transmission and LTP in the dentate lateral perfor-
ant path. OFQ-induced inhibition of synaptic transmission appears to be caused by a reduction in excitability of dentate granule cells, which results from granule cell hyperpolarization via OFQ-activated K$^+$ currents. In addition, OFQ strongly inhibits NMDA receptor–mediated synaptic and membrane currents. This effect, in combination with granule cell hyperpolarization, may have significant contribution to the inhibition of LTP by OFQ.

**OFQ-induced inhibitory effects on dentate synaptic transmission**

Similar to its action in the CA1 region, OFQ inhibits synaptic transmission in the dentate gyrus. Interestingly, we found that OFQ inhibited the population spike amplitude in a dose-dependent manner but had no significant effect on the EPSP slope. This is in contrast to our previous finding in the CA1 area, where both population spike and EPSP slope were depressed by the peptide (Yu et al. 1997). The insensitivity of OFQ-induced inhibition to opioid receptor antagonists suggests that the actions of OFQ are unlikely mediated by opioid receptors, which is consistent with previous studies (Abdula and Smith 1997; Faber et al. 1996; Knofflach et al. 1996; Liebel et al. 1996; Nicol et al. 1996; Vaughan et al. 1997; Vaughan and Christie 1996; Wang et al. 1996). Because in the hippocampus the major type of glutamate receptors involved in excitatory synaptic transmission is the AMPA receptor (Watkins and Evans 1981), the absence of significant inhibition on EPSP slope by OFQ may be explained by its selective effect on NMDA but not AMPA receptor–mediated EPSCs in the dentate.

**Possible postsynaptic mechanisms underlying OFQ-induced inhibition of synaptic transmission**

OFQ reportedly has various inhibitory actions in different brain regions through both presynaptic and postsynaptic mechanisms. For instance, OFQ is able to inhibit K$^+$-evoked glutamate release from rat cerebrocortical slices (Nicol et al. 1996), depress glutamatergic transmission in the neonatal

**FIG. 5.** OFQ selectively inhibits N-methyl-d-aspartate (NMDA) receptor–mediated excitatory postsynaptic currents (NMDA EPSCs). A: OFQ suppressed NMDA EPSCs and had no effect on EPSCs mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPA EPSCs) in dentate granule cells. Cells were held at −70 mV. NMDA EPSCs were recorded with K$^+$- or Cs$^+$-filled micropipettes in Mg$^{2+}$-free ACSF containing 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 μM (-)-bicuculline methiodide (BMI). AMPA EPSCs were recorded in the presence of 40 μM d(-)-2-amino-5-phosphonopentanoic acid [d(-)-AP-5] and 50 μM BMI (c). Synaptic currents (from left to right) were recorded before the application of OFQ (baseline) in the presence of OFQ (0.5 μM for a and c; 1 μM for b) and after washout of the peptide. OFQ reduced NMDA EPSCs by 23% in the cell recorded with K$^+$-filled pipette (a) and by 40% in another cell recorded with Cs$^+$-filled pipette (b). In contrast, the peptide did not affect AMPA EPSCs. Calibration bars: 50 pA, 200 ms for NMDA EPSCs and 200 pA, 50 ms for AMPA EPSCs. B: reduction of NMDA-mediated EPSCs by OFQ was concentration dependent. Effect of 0.5 μM OFQ was not affected by the opioid receptor antagonist naloxone (10 μM) or by a selective κ receptor antagonist nor-BNI (1 μM). In contrast to a significant inhibition of NMDA EPSC induced by OFQ (n = 16, *P < 0.01), the inactive analogue of OFQ, des-Phe$^1$-OFQ (1 μM), synthesized by eliminating the N-terminal phenylalanine residue, did not inhibit NMDA EPSCs (n = 5).
OFQ-induced inhibition of dentate LTP and NMDA currents in the dentate granule cells

LTP at the lateral perforant path-dentate granule cell synapses, which is NMDA receptor dependent (Colino and Maenko 1993; Hanse and Gustafsson 1992), was significantly inhibited by OFQ. Such effect was not mediated through opioid receptors as indicated by insensitivity of OFQ-induced inhibition to opioid receptor antagonists. There are several mechanisms that could be responsible for the inhibition of LTP. OFQ may suppress LTP by reducing presynaptic excitatory transmitter release, inhibiting the excitability of postsynaptic neurons or suppressing some key mechanisms required for the induction of LTP such as activation of NMDA receptors/channels. Our results showed that OFQ selectively suppressed NMDA but not AMPA receptor–mediated EPSCs. In addition, OFQ significantly attenuated the inward currents evoked by focal application of NMDA to the granule cells. These results strongly suggest that OFQ can directly act on granule cells to inhibit the currents through NMDA receptors/channels. This mechanism as well as hyperpolarization of granule cells may both contribute to the inhibition of LTP.

The detailed mechanism for inhibition of NMDA currents remains to be determined. Under physiological conditions, hyperpolarization of postsynaptic neurons can enhance Mg$^{2+}$-blockade of NMDA channels, resulting in attenuation of
NMDA currents. It is therefore possible that reduction of NMDA currents observed here was a secondary action caused by OFQ-induced cell membrane hyperpolarization and/or K⁺ conductance increases. However, NMDA currents were recorded under voltage-clamp conditions in Mg²⁺-free perfusion medium. Mg²⁺ block of NMDA channels could be significantly relieved under such conditions, as demonstrated in our previous study (Xie and Lewis 1997). Moreover, when using Cs⁺-filled pipettes to block K⁺ conductance in granule cells, we still observed significant inhibition of NMDA EPSCs by OFQ, and the degree of inhibition was comparable with that observed with K⁺-filled pipettes. Taken together, it seems unlikely that the reduction of NMDA currents after OFQ perfusion was caused by hyperpolarization and/or K⁺ conductance increases in granule cells. Alternatively, OFQ may have a direct effect on post-synaptic NMDA receptor/channel complex, or affect intraacellular signaling pathways that regulate the activity of the NMDA receptors/channels. Our recent study indicates that μ opioids can inhibit NMDA receptor-mediated currents through suppression of the cAMP cascade (Xie and Lewis 1997). Because the ORL1 receptor also negatively couples to adenylyl cyclase, it is likely that OFQ may inhibit NMDA current through a similar mechanism. This possibility is currently under investigation.

The authors thank Dr. Qi-Sheng Chen for conducting some of the experiments, Dr. Christopher J. Evans and J. Fein for supply of the peptides used in this study, and Drs. Time Hales, Kabir Lutfy, and Niall Murphy for comments on an earlier version of this manuscript.

This study was supported by National Institute of Drug Abuse Grants DA-05010 and R29 DA-08571 to C. W. Xie. Present address of T.-P. Yu: Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125.

Address for reprint requests: C.-W. Xie, Dept of Psychiatry and Biobehavioral Sciences, 760 Westwood Plaza, University of California, Los Angeles, CA 90024-1759.

Received 9 February 1998; accepted in final form 3 June 1998.

REFERENCES


