Orphanin FQ Suppresses NMDA Receptor-Dependent Long-Term Depression and Depotentiation in Hippocampal Dentate Gyrus

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Abstract

We reported previously that orphanin FQ (OFQ) inhibited NMDA receptor-mediated synaptic currents and consequently suppressed induction of long-term potentiation (LTP) in the hippocampal dentate gyrus. This study examines the effect of OFQ on several other forms of long-term synaptic plasticity in the lateral perforant path of mouse hippocampal dentate gyrus. (1) Long-term depression (LTD): a low frequency stimulation (1 Hz, 15 min) applied to the lateral perforant path induced a long-lasting reduction in the dentate field potentials in slices from 22- to 30-day-old mice. This LTD was sensitive to the NMDA receptor blocker D-AP5, and could be significantly attenuated by bath application of OFQ (1 µM, 25 min). (2) Primed LTD: induction of LTD in slices from 50- to 65-day-old mice required a priming procedure consisting of multiple high frequency stimulus trains delivered in the presence of D-AP5 before the low-frequency stimulation. OFQ applied during the low-frequency stimulation, but not during the priming trains, blocked induction of primed LTD. (3) Depotentiation: high-frequency train-induced dentate LTP could be reversed by a subsequent low-frequency stimulation. This depotentiation was also attenuated by either OFQ or D-AP5 applied during low-frequency stimulation. These results, together with our previous findings, suggest that OFQ inhibits bidirectional changes in synaptic strength in the dentate; and its multiple actions on NMDA receptor-dependent, long-term synaptic plasticity might work in tandem to regulate hippocampus-dependent learning and memory.

Introduction

Orphanin FQ (OFQ), also called nociceptin, is a recently identified endogenous heptadecapeptide that activates the opioid receptor-like (ORL1) receptor in the brain and reportedly induces hyperalgesia and hypolocomotion in rodents (Meunier et al. 1995; Reinscheid et al. 1995). The ORL-1 receptors are highly expressed in the limbic system regions, such as the hippocampus and amygdala (Lachowicz et al. 1995; Anton et al. 1996). Because these brain regions, particularly hippocampus, are known to be important for memory formation and certain types of learning, there has been growing interests in examining the function of the OFQ/ORL1 system in these areas with regards to the modulation of learning and memory. Our earlier studies showed that application of OFQ strongly inhibited induction of long-term potentiation (LTP) in area CA1 and dentate gyrus of rat hippocampal slices (Yu et al. 1997). Accordingly, an in vivo study demonstrated impairment of spatial learning following intrahippocampal injection of OFQ in rats (Sandin et al. 1997). These findings indicated that OFQ could modulate hippocampus-dependent learning and memory negatively, and suppression of activity-dependent, long-term synaptic plasticity might underlie its action. This no-
tion was supported further by a gene targeting study demonstrating facilitation of CA1 LTP and improved learning and memory in mice lacking ORL-1 receptors (Manabe et al. 1998). Effort has been made to further elucidate the cellular mechanisms underlying OFQ-induced learning deficits. Our most recent study revealed that OFQ inhibited NMDA receptor-mediated synaptic currents strongly via a postsynaptic mechanism in the dentate granule cells (Yu and Xie 1998). Because LTP in the dentate perforant path, both the lateral and medial division, is NMDA receptor-dependent (Colino and Malenka 1993), this finding provided a possible underlying mechanism for impairment of dentate LTP. It also raised several interesting questions. For example, can OFQ suppress other forms of activity-dependent synaptic plasticity that are potentially important for information storage in the hippocampus? If so, is the action of OFQ contingent on the NMDA receptor dependence of the plasticity, that is, does OFQ selectively inhibit those requiring activation of NMDA receptors? This study attempted to address these questions by examining the effect of OFQ on low-frequency stimulation-induced long-term depression (LTD) and depotentiation at the lateral perforant path-dentate granule cell synapse in mouse hippocampal slices.

LTD is an activity-dependent, long-lasting decrease in synaptic efficacy (Bear and Malenka 1994). In brain slices from young animals, homosynaptic LTD can be induced by a brief period of low-frequency stimulation (LFS) at 1–10 Hz in all three hippocampal subregions—CA1 (Dudek and Bear 1992; Mulkey and Malenka 1992), CA3 (Derrick and Martinez 1996; Kobayashi et al. 1996), and dentate gyrus (O’Mara et al. 1995; Wang et al. 1998). The similar LFS protocol, when applied after induction of LTP, can produce depotentiation, a rapid reversal of potentiation to the baseline level (Wagner and Alger 1995; Doyle et al. 1997). Both LTD and depotentiation are considered useful models for experience-dependent persistent reduction in synaptic strength, which may operate together with synaptic potentiation during information processing in the brain (Xu et al. 1997, 1998). The underlying mechanisms for both phenomenon, however, have not been well understood. At the Schaffer collateral-CA1 synapse induction of LTD or depotentiation is known to require a moderate rise in postsynaptic Ca2+ following activation of NMDA receptors (Cummings et al. 1996) or metabotropic glutamate receptors (mGLuRs) (Oliet et al. 1997). Similarly, LTD or depotentiation in the dentate medial perforant path is also Ca2+ dependent, but the source of the Ca2+ rise remains controversial, with evidence for the involvement of NMDA receptors (Desmond et al. 1991; Thiels et al. 1996), metabotropic glutamate receptors (mGluR) (O’Mara et al. 1995), voltage-gated Ca2+ channels (Wang et al. 1997; Wang et al. 1997) and release of Ca2+ from intracellular stores (Wang et al. 1997). Little is known regarding the LFS-induced homosynaptic LTD and depotentiation in the lateral perforant path. An earlier study reported that LTD and depotentiation could be induced in this pathway by a 1-Hz LFS in slices from 4- to 6-week-old mice (Brandon et al. 1995) but mechanisms for their induction and modulation remain largely unexplored to date.

Another plasticity model examined in the present study is the priming of LTD. It is well known that induction of LTD in area CA1 is age dependent. Although depotentiation can be induced readily by LFS in mature preparation from >30-day-old animals, successful induction of LTD in vitro has been limited to slices from younger animals (Mulkey and Malenka 1992; Mulkey et al. 1994; Wagner and Alger 1995). Application of a brief period of high-frequency stimulation (HFS, 30–100 Hz) before the LFS reportedly “primes” the induction of LTD in area CA1 of mature slices (Wexler and Stanton 1993; Hollan and Wagner 1998). It is unclear whether similar protocols would also facilitate LTD in the dentate. Furthermore, the priming of LTD by prior synaptic activity is consistent with the concept of metaplasitcity, that is, certain patterns of cellular or synaptic activity can change the ability of synapses to induce subsequent plasticity, whereas not necessarily altering the efficacy of normal synaptic transmission (Abraham and Bear 1996). It is worth further investigating whether this type of plasticity can also be modulated by OFQ in the dentate gyrus.

**Materials and Methods**

**SLICE PREPARATION AND EXTRACELLULAR RECORDINGS**

Transverse hippocampal slices (500 µm) were prepared from 22- to 65-day-old male C57 black 6 mice and maintained in a holding chamber with oxygenated artificial cerebrospinal fluid (ACSF) at 30 ± 1°C. The ACSF contained 120 mM NaCl, 25 mM NaHCO3, 3.3 mM KCl, 1.23 mM NaH2PO4, 2 mM CaCl2, 1 mM MgSO4, and 10 mM d-glucose (pH 7.4).
After at least 1 hr incubation in the holding chamber, the slice was transferred to a 2-ml submerged chamber for recording, where it was perfused continuously with oxygenated, warm (30 ± 1°C) ACSF at a rate of 2–3 ml/min throughout experiments.

The lateral perforant path was stimulated via a sharpened monopolar tungsten electrode positioned in the outer third of the dentate molecular layer. Field excitatory postsynaptic potentials (fEPSPs) evoked by constant-current stimulus pulses (0.1 msec, 30–300 µÅ) were recorded extracellularly from the outer molecular layer using a glass micropipette filled with 2M NaCl (1–8 M). Paired pulse tests (50–100 msec intervals) were conducted to ensure that responses evoked were from the lateral perforant path, which showed clear paired pulse facilitation in contrast to the paired pulse depression in the neighboring medial perforant path (McNaughton 1980).

EXPERIMENTAL DESIGN

Field potentials were evoked at various stimulus intensities to determine the stimulation-response relationship at the beginning of each experiment. The stimulus intensity that evoke 40%–50% of the maximum EPSP slope was then chosen for the test pulse and LFS or HFS. The test pulses were delivered at 0.01 Hz throughout the experiment for baseline recording and monitoring the changes in field potentials after the induction of plasticity by LFS or HFS. An LFS protocol (1 Hz, 15 min) was used for induction of LTD. In mature slices from 50- to 65-day-old mice, a priming protocol (Hollan and Wagner 1998) was applied before the LFS to facilitate LTD induction. The protocol included two sets of stimulation, each consisting of three high-frequency trains (HFS, 100 Hz/1 s) at 20 sec intervals, with 15 min between sets. The priming trains were delivered in the presence of the NMDA receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, 50 µM) to prevent LTP, and were followed by a LFS 40 min later to induce LTD. In depotentiation experiments, LTP was first induced by two sets of HFS as was used for priming, except that no D-AP5 was applied during the HFS. After observing a stable LTP for 40 min, the LFS was applied to reverse the potentiation.

OFQ (Phoenix Pharmaceuticals, Mountain View, CA) or the NMDA receptor antagonist D-AP5 was bath-applied at indicated concentrations, starting 10 min before the train stimulation (LFS or HFS as noted) and continuing through the train stimulation for a total period of 25 min. Applied drugs were washed out immediately after completion of the train stimulation. Changes in synaptic strength were quantified 30 min after the train in drug-free ACSF and expressed as percent changes from the pre-drug baseline level.

DATA ANALYSIS

In LTD experiments, changes in fEPSP slopes were quantified 30 min after the LFS and expressed as percent reductions from the average baseline level collected for 10 min before the LFS. In depotentiation experiments, HFS-induced LTP was assessed 30 min after the last train, which was set as the pre-depotentiation level; the remaining LTP after the depotentiation was determined 30 min after the LFS; and all changes in fEPSP slopes were expressed as percent changes from the average baseline level.

The recorded responses were amplified, digitized and stored on computer disks for off-line analysis. The group data were presented as mean ± S.E., with n value representing number of slices, one from each animal, for each group. Data were first subjected to a one-way analysis of variance (ANOVA) to test for overall statistical significance, and comparisons between groups were then made by student’s t-tests. Statistical significance was defined as P<0.05.

Results

SUPPRESSION OF LTD BY ORPHANIN FQ AND D-AP5

In slices from 22- to 30-day-old mice, an LFS (1 Hz, 15 min) was applied to the lateral perforant path to induce LTD in the dentate gyrus. As demonstrated in Figure 1, this LFS protocol produced a significant reduction in the fEPSP slope, which lasted for at least 30 min in control slices (31 ± 3% reduction, n = 12). Bath-applied OFQ at 1 µM for 25 min did not affect the baseline responses or the initial reduction of fEPSP slopes immediately after the LFS. The LFS-induced depression, however, recovered much faster in OFQ-treated slices, leaving only 8 ± 4% reduction from the baseline 30 min after the LFS (n = 12, P < 0.01 as compared with the control LTD). The NMDA receptor antagonist D-AP5 was applied in a similar manner to another group of slices (50 µM, 25 min). LTD in this group...
of slices was also substantially suppressed (5 ± 3% reduction 30 min after the LFS, \(n = 5\), \(P < 0.01\) as compared with the control LTD), though its initial phase was not completely eliminated by D-AP5 (Fig. 1).

**THE EFFECT OF OFQ AND D-AP5 ON PRIMING AND PRIMED LTD**

Whereas the LFS protocol routinely induced LTD in young slices, it produced little depression in slices from 50- to 65-day-old mice (1 ± 3%, \(n = 6\)). In those mature slices, a priming protocol was applied to facilitate LTD induction, which consisted of two sets of HFS followed 40 min later by the LFS. As shown in Figure 2, A and B, a significant LTD could be obtained in mature slices treated with this priming procedure (28 ± 4%, \(n = 8\), \(P < 0.01\) as compared with the unprimed control group). Because the NMDA receptor antagonist D-AP5 (50 µM) was routinely applied during the priming trains to prevent LTP, apparently the priming process occurring during the HFS was NMDA receptor-independent. When the perfusion of D-AP5 was extended to cover the period of LFS (Fig. 2A,C), however, the induction of LTD was suppressed (0 ± 2%, \(n = 5\)).

Interestingly, OFQ displayed actions similar to D-AP5 on priming and primed LTD. When 1 µM OFQ was applied during the high-frequency priming trains (Fig. 3A,B), the amount of primed LTD was 29 ± 2% (\(n = 5\)), indistinguishable from that in the control slices (28 ± 2%, \(n = 8\), \(P = 0.8\)). In contrast, when OFQ was applied during the LFS (Fig. 3A,C), the primed LTD was significantly inhibited (4 ± 4%, \(n = 6\), \(P < 0.01\) as compared with the controls). Therefore, similar to D-AP5, OFQ did not block the priming event during HFS, nevertheless it suppressed the final induction of LTD during LFS in primed slices.

**INHIBITION OF LFS-INDUCED DEPOTENTIATION BY OFQ AND D-AP5**

Multiple HFS to the lateral perforant path induced a long-lasting LTP in control slices from 50- to 65-day-old mice. As shown in Figure 4, the LTP in control slices (\(n = 6\)) was 56 ± 4% above the baseline 30 min after the HFS and remained at 47 ± 3% by the end of experiments (85 min after the HFS), with <10% decay in nearly 1 hr. Application of an LFS after induction of LTP was able to cause a rapid and complete reversal of this otherwise lasting LTP. In the depotentiated slices there was only a 5 ± 3% (\(n = 7\)) potentiation remaining 30 min after the LFS (85 min after the HFS), which was significantly smaller than both the pre-depotentiation level in the same slices (57 ± 9%, 30 min after the HFS) and the remaining LTP in control slices 85 min after the HFS (\(P < 0.01\) for both comparisons).
OFQ (1 µM) or D-AP5 (50 µM), applied during the LFS, significantly attenuated the depotentiation (Fig. 4). In slices treated with either drug, only a transient reduction in fEPSP slopes was observed after the LFS, which mostly recovered within 20 min. Thirty min after the LFS the remaining LTP was $39 \pm 8\%$ in OFQ-treated slices ($n = 7$) and $31 \pm 9\%$ in D-AP5-treated slices ($n = 4$). Both values were not significantly different from the control LTP at the corresponding time point ($47 \pm 3\%$), but substantially greater than the remaining LTP in the depotentiation group ($5 \pm 3\%$, $P < 0.01$).

**Discussion**

**BIDIRECTIONAL MODIFICATION OF SYNAPTIC STRENGTH BY OFQ IN THE DENTATE GYRUS**

Activity or experience-dependent bidirectional changes in the strength of synaptic connections...
are believed to underlie learning and memory in the mammalian brain. The direction and magnitude of such changes often are contingent on previous history of synaptic activity and current activity patterns. Behavioral stress has been found to inhibit induction of LTP (Shors et al. 1989; Diamond and Rose 1994) but facilitate LTD in area CA1 of adult rat hippocampus (Xu et al. 1997). Exploration of a new, nonstressful environment induces a persistent reversal of hippocampal LTP in freely moving rats (Xu et al. 1998). Therefore, long-lasting decreases in synaptic efficacy can occur in the brain of behaving animals. Such decreases have been proposed to be crucial for preserving the capacity for change at selected synapses, allowing the detection and storage of new information in the neuronal network (Bear and Malenka 1994; Abraham and Bear 1996). In this study OFQ strongly inhibited LTD and depotentiation in the dentate gyrus. Combined with the previous finding that this peptide inhibited LTP induction in the dentate and area CA1 (Yu and Xie 1998), our results indicated that OFQ could suppress both enhancing and suppressive forms of synaptic plasticity, thereby modifying synaptic strength in a bidirectional manner in the hippocampal circuitry. By inhibiting multiple forms of long-term synaptic plasticity at selected synapses, OFQ would be expected to efficiently interfere information processing and storage at these synapses and consequently disrupt hippocampus-dependent learning and memory, as shown in previous behavioral tests (Sandin et al. 1997; Manabe et al. 1998).

To better understand the integrative role of

Figure 3: OFQ inhibits primed LTD but not the priming process in mature slices. (A) Sample fEPSPs recorded before (1) and 30 min after the LFS (2) in slices treated with OFQ during either the HFS or LFS. Calibration bars, 2 mV, 10 msec. (B) Application of OFQ during the HFS did not affect the priming effect of HFS on LTD induction. (C) OFQ applied during the LFS significantly suppressed the final induction of LTD in primed slices. The priming protocol, as described in the Fig. 2, was applied in the presence of D-AP5 for all slices. n = 5–8 for each group.

Wei and Xie
OFQ in the modulation of hippocampal function, it may be necessary to consider the proposed role of OFQ as a novel "anti-opiate" peptide in the brain (Mogil et al. 1996). Besides its well documented anti-morphine action in behavioral tests (Mogil et al. 1996; King et al. 1998; Tian et al. 1998), OFQ apparently also has opposite effects to endogenous opioids acting on μ or δ receptors in the hippocampus. In contrast to the suppressive effect of OFQ on both LTP and LTD, the endogenous opioids released from the lateral perforant path, mainly enkephalins (Gall et al. 1981), have been shown to facilitate dentate LTP (Bramham et al. 1988; Xie and Lewis 1991, 1995). In addition, activation of enkephalin-containing CA1 perforant projections or local opioid-containing interneurons are believed responsible for a naloxone sensitive LTD in area CA1 (Francesconi et al. 1997). Considering the highly abundant presence of ORL-1 receptors (Lachowicz et al. 1995; Anton et al. 1996; Florin et al. 1997) as well as μ and δ opioid receptors (Mansour et al. 1987) in the hippocampus, the interaction between these two peptide systems could have significant influence on the modulation of hippocampal plasticity. In this context, the bi-directional action of OFQ, when operating together with endogenous μ/δ opioids or other plasticity enhancers, may provide an efficient means for adjusting the direction and magnitude of plastic changes in synaptic connection, thereby increasing the flexibility and maximal capacity of the system as a whole.

NMDA RECEPTOR-DEPENDENT LTD
AND DEPOTENTIATION IN THE DENTATE GYRUS

Previously filed potential studies have shown that LFS-induced LTD or depotentiation at the me-
In the present study, LFS-induced LTD and depotentiation at the lateral perforant path-dentate granule cell synapse were significantly attenuated by 50 µM D-AP5, which was sufficient for complete blockade of NMDA receptor-mediated synaptic currents in dentate granule cells (Xie et al. 1992). These results indicate that, different from the medial perforant path, LFS-induced synaptic depression in the lateral perforant path seems largely dependent on Ca\(^{2+}\) influx through NMDA receptor channels even under conditions of field potential recordings. Anatomical (Steward 1976), biochemical (Gall et al. 1981; Fredens et al. 1984), and electrophysiological (McNaughton and Barnes 1977) evidence has long demonstrated that the lateral and medial divisions of the perforant path are two distinct pathways. LTP induction in these two pathways is known to be differentially regulated by endogenous opioids, with naloxone blocking LTP in the lateral but not the medial one (Bramham et al. 1988; Xie and Lewis 1991). Therefore, it is not surprising that induction of LTD or depotentiation in these two pathways can display different degree of NMDA receptor dependence. On the other hand, D-AP5 significantly reduced, but did not completely eliminate, LTD and depotentiation in this study. We cannot exclude the possibility that some NMDA receptor-independent mechanisms are also involved, to a minor extent, in synaptic depression in the lateral perforant path. Studies in area CA1 have shown that induction of mGluR-dependent LTD can be facilitated by altering the concentrations and ratio of extracellular Ca\(^{2+}\) and Mg\(^{2+}\) (Oliet et al. 1997). It will be of interest to examine in future studies whether under different experimental conditions the potential NMDA receptor-independent mechanisms can be exacerbated for further analysis at the lateral perforant path synapses.

**POSSIBLE MECHANISMS FOR THE INHIBITORY ACTION OF OFQ ON SYNAPTIC DEPRESSION**

Because activation of NMDA receptors proved essential for synaptic depression in the lateral perforant path, suppression of NMDA receptor function might constitute a major underlying mechanism for the inhibitory action of OFQ on LTD and depotentiation in this pathway. The effect of OFQ was completely parallel with the action of D-AP5 in several different plasticity tests. Both OFQ and D-AP5 inhibited LTD and depotentiation but not the priming of LTD. The selective effect of OFQ on D-AP5-sensitive plasticity was consistent with the previous finding that OFQ strongly inhibited NMDA receptor-mediated synaptic currents in dentate granule cells (Yu and Xie 1998). Together, these results indicate that blockade of currents through NMDA receptor channels on the postsynaptic neurons is the most likely common mechanism for the inhibitory action of OFQ on multiple forms of synaptic plasticity in the dentate gyrus.

It should be noted, however, that inhibition of NMDA receptor currents may not be the sole mechanism responsible for suppression of LTD or depotentiation by OFQ. There are other possible mechanisms, such as OFQ-induced hyperpolarization of dentate granule cells following activation of a K\(^{+}\) conductance (Yu and Xie 1998) and inhibition of voltage-activated Ca\(^{2+}\) channels (Knoflach et al. 1996; Abdulla and Smith 1997). Both effects of OFQ may reduce depolarization-induced Ca\(^{2+}\) influx needed for induction of LTD or depotentiation. In addition, activity of cAMP-dependent protein kinase (PKA) has been found necessary for LTD expression in the lateral perforant path (Brandon et al. 1995). Inhibition of the intracellular cAMP/PKA cascade by OFQ (Meunier et al. 1995; Reinscheid et al. 1995) may be another contributing factor for the suppression of LTD.

A different type of plasticity examined in this study was the priming of LTD. LFS alone was found to induce little LTD in the lateral perforant path of adult animals, suggesting that LTD induction in this pathway possesses similar developmental properties as observed in area CA1. By adopting a HFS protocol used previously in CA1 (Hollan and Wag-
ner 1998), we successfully primed LTD induction in mature slices, indicating that the dentate synapses are also capable of showing this type of metaplasticity. Both OFQ and D-AP5 significantly inhibited primed LTD when applied during the LFS, but neither blocked the priming itself when applied only during the high-frequency priming trains before the LFS. Apparently, the priming mechanisms activated during the HFS were NMDA receptor independent, nor they were affected by OFQ. Using different priming procedures, previous studies have proposed several underlying mechanisms for priming of LTD in the dentate or CA1, such as activation of group 2 mGluR (Manahan-Vaughan 1998), involvement of protein kinase C (Wang et al. 1998), and reduction of GABAergic synaptic inhibition (Wagner and Alger 1996). The critical mechanisms involved in our priming protocol remain to be determined.

In summary, OFQ displayed strong inhibitory action on LFS-induced LTD and depotentiation at the lateral perforant path-dentate granule cell synapse of hippocampal slices. OFQ also suppressed HFS-primed LTD but did not affect the priming process itself. In all these tests, the action of OFQ was completely parallel with the NMDA receptor antagonist D-AP5, suggesting that blockade of NMDA receptor-mediated synaptic currents may be the common mechanisms underlying the inhibitory action of OFQ on multiple forms of synaptic plasticity. The ability of OFQ to alter both LTP and LTD/depotentiation may enable the peptide to regulate synaptic plasticity in a bidirectional manner, and thereby efficiently affect hippocampus-dependent learning and memory.

Acknowledgments

This work is supported by National Institutes of Health grants DA08571 and DA05010 (CWX).

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Received July 8, 1999; accepted in revised form August 22, 1999.


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Access the most recent version at doi:10.1101/Im.6.5.467

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