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Orphanin FQ Inhibits Synaptic Transmission and Long-Term Potentiation in Rat Hippocampus

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ABSTRACT: It is known that opioid peptides acting on opioid receptors can modulate hippocampal synaptic functions. Although a novel member of the opioid receptor family, ORL_1 receptors, that displays high-sequence homology with classical opioid receptors is abundant in the hippocampus, little is known regarding its role in synaptic function. The present study was designed to investigate whether activation of the ORL_1 receptor by its natural ligand, orphanin FQ, could modulate synaptic transmission and synaptic plasticity in the hippocampus. The actions of orphanin FQ in the CA1 and dentate gyrus were examined by field potential recordings in response to stimulation of Schaffer collaterals and perforant path, respectively. Our results showed that orphanin FQ, but not the inactive analog des-Phe^1-orphanin FQ, reduced both the slope of the excitatory postsynaptic potentials and population spike amplitude. The inhibitory effect of orphanin FQ is dose dependent and probably involves a presynaptic mechanism, as suggested by the significantly increased paired-pulse facilitation evoked in the presence of orphanin FQ. In addition, orphanin FQ was found to inhibit the induction of long-term potentiation at the Schaffer collateral–CA1 synapse. These results demonstrate that orphanin FQ can function as an inhibitory modulator regulating synaptic transmission and synaptic plasticity in the hippocampus, suggesting that activation of ORL_1 receptors may play an important role in synaptic plasticity involved in learning and memory. *Hippocampus 7:88–94, 1997.*

KEY WORDS: ORL_1 receptor; field potential recording; des-Phe^1-orphanin FQ; paired-pulse facilitation; synaptic plasticity

INTRODUCTION

In the hippocampus, it is clear that activation of opioid receptors such as mu (μ), delta (δ), and kappa (κ) receptors can modulate synaptic transmission and synaptic plasticity in the dentate gyrus (Bramham et al., 1991; Xie and Lewis, 1991, 1995a,b; Terman et al., 1993, 1994; Wagner et al., 1993), CA1 (Wimpey et al., 1990; Watson and Lanthorn, 1993; Jones et al., 1994), and CA3 (Derrick et al., 1992; Weisskopf et al., 1993; Caudle et al., 1994). In these systems, opioid peptides may function as neurotransmitters or modulators to regulate excitability of neurons (Moises and Walker, 1985; Bramham, 1992), which has been suggested to play a role in a process associated with learning and memory (Gallagher et al., 1983; Collier and Routtenberg, 1984). Our previous immunohistochemical study has shown that a novel member of the opioid receptor family, the ORL_1 receptor, is expressed in relatively high abundance in the rat hippocampus (Anton et al., 1996). This receptor is closely homologous to the μ, δ, and κ receptors, but does not bind with appreciable affinity to the classical opioid ligands (Bunzow et al., 1994; Chen et al., 1994; Fukuda et al., 1994; Keith et al., 1994; Marchese et al., 1994; Mollereau et al., 1994; Wick et al., 1994; Lachowicz et al., 1995).

Recently, an endogenous ligand for the ORL_1 receptor, orphanin FQ was isolated and shown to have hyperalgesic rather than analgesic properties (Meunier et al., 1995; Reinscheid et al., 1995), suggesting an anti-opioid role of this peptide in nociceptive processing. However, little is known regarding the synaptic function of orphanin FQ. The present study was designed to investigate the actions of orphanin FQ in the rat hippocampus and to examine whether orphanin FQ also plays an anti-opioid role in hippocampal synaptic functions. We focused on the CA1 and dentate gyrus because of the abundant distribution of the ORL_1 receptor in both regions (Anton et al., 1996). Our results provide the first electrophysiological evidence for an inhibitory effect of orphanin FQ on synaptic transmission and synaptic plasticity such as long-term potentiation (LTP) in the rat hippocampus.

MATERIALS AND METHODS

Slice Preparation

Transverse hippocampal slices (500 μm) were prepared from male Sprague-Dawley rats (24–40 days old) and maintained in a holding chamber with oxygenated artificial cerebrospinal fluid (CSF) at 32°C. The artificial CSF contained (in mM) NaCl, 120; $NaHCO_3$, 25; KCl,

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3.3; NaH_2PO_4, 1.23; $CaCl_2$, 1.8; $MgCl_2$, 1.2; and D-glucose, 10. After a 1-h incubation in the holding chamber, the slice was transferred to a submersion recording chamber perfused with artificial CSF at a rate of 2–3 ml/min.

Electrophysiology

Extracellular field potentials were recorded with glass micropipettes filled with 2 M NaCl. In the dentate gyrus, evoked responses in the granule cell layer were recorded in response to stimulation of the lateral perforant path through a stimulating electrode positioned in the outer molecular layer. In CA1 region, Schaffer collaterals were stimulated, and evoked responses were recorded from the pyramidal cell layer and the dendritic layer (stratum radiatum). Constant stimulus pulses (0.1 ms, 0.06–0.4 mA) were delivered by a stimulator (Grass Model S88) through a sharpened monopolar electrode. The stimulus intensity that evoked 50–60% of the maximum response was chosen as a test pulse to obtain baseline responses and also for subsequent train stimuli in LTP experiments.

Paired-pulse stimuli that only induced excitatory postsynaptic potentials (EPSPs) without triggering population spikes (0.07–0.1 mA, 0.1 ms in duration with an interstimulus interval of 50 ms) were used to examine paired-pulse facilitation. In the presence of orphanin FQ, the stimulus intensity was adjusted so that the amplitude of the response to the first pulse matched the amplitude of the control response. The amount of paired-pulse facilitation was expressed as the percent increase in the second response amplitude relative to the first one taken as 100%.

In LTP experiments, stable baseline responses evoked by test pulses (as described above) in artificial CSF were first collected. Orphanin FQ was then bath-applied to the slices for 10 min, during which responses evoked by test pulses were recorded. Immediately following the 10-min peptide perfusion, a high-frequency stimulation was applied in the presence of orphanin FQ to induce LTP. The high-frequency stimuli consisted of ten brief trains (4 pulses at 100 Hz) with an intertrain frequency of 5 Hz and were applied at adjusted stimulus intensity that obtained the same population spike amplitude as the baseline. The peptide was washed off immediately after trains. Post-train responses evoked by the same test pulses that evoked baseline responses were examined during a 30-min washing. The baseline response in artificial CSF was defined as 100%. The amount of LTP was expressed as a percentage of change in the post-train population spike or EPSP slope, relative to the baseline response.

The recorded responses were amplified, digitized, and stored on computer disk for off-line analysis. Data were presented as mean ± SEM (standard error of mean). A paired Student *t*-test was used to compare the paired-pulse facilitation in the absence and presence of orphanin FQ. Tetanus-induced facilitation in the control and peptide-treated groups was compared using an unpaired Student *t*-test, provided that the measures were obtained separately from two groups of slices. Difference was considered significant at the level of $P < 0.05$.

Peptide Synthesis

Both orphanin FQ (FGGFTGARKSARKLANQ) and des-Phe^1-orphanin FQ were synthesized on 2-chlorotrityl resin (Anaspec Inc.) using standard Fmoc procedures (Hockfield et al., 1993). Purity was achieved with reverse-phase, high-performance liquid chromatography (HPLC), and fast atom bombardment mass spectroscopy (FAB) was used to determine structural homogeneity. The final peptide products were lyophilized and stored at −20°C. Both peptides were dissolved in deionized water and prepared as 5 mM stock solutions stored at −20°C. To ensure the potency, a stock solution was divided into a number of vials, and each vial contained solution for only one experiment. Stock solutions were diluted in artificial CSF prior to application.

Receptor Binding Assays

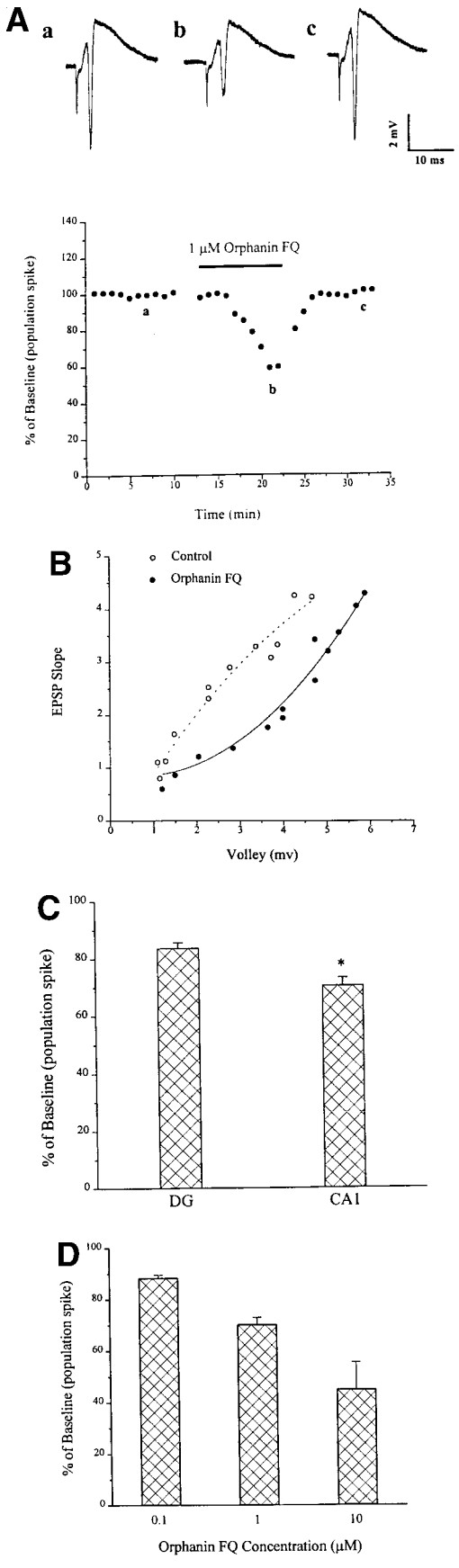
Receptor binding was assayed with P2 membrane preparations. Rat brains (minus cerebellum) were homogenized at 4°C in 50 mM Tris, 10% sucrose, 1 mM PMSF using a Polytron at setting 4 and centrifuged at 1,000 *g* for 10 min. The pellet was resuspended, homogenized, and re-centrifuged. Both supernatants were pooled and centrifuged at 10,000 *g* for 30 min. The membrane pellet was resuspended in the same buffer and stored at −70°C. The protein concentration was determined using the BCA protein assay (Pierce). [Tyr^{14}]-orphanin FQ was iodinated with ^{125}I using standard methods and purified by reverse phase HPLC (Maidment et al., 1989).

Binding assay was performed by adding membranes (~40 μg protein), ^{125}I labeled [Tyr^{14}]-orphanin FQ (~100,000 cpm), and competitive ligand (orphanin FQ, or des-Phe^1-orphanin FQ) to a final volume of 0.1 ml [50 mM HEPES, 10 mM NaCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 2% bovine serum albumin, 0.025% bacitracin] and incubated at 4°C for 60 min. After the incubation, 1.0 ml of binding buffer was added to the membrane suspension and centrifuged at 10,000 *g* for 30 s. Bound ^{125}I-labeled orphanin FQ in the pellets was measured using a gamma counter (Micromedic system 4/600).

RESULTS

Orphanin FQ Inhibits Synaptic Transmission in Both CA1 and Dentate Gyrus

The role of orphanin FQ in synaptic transmission was investigated by examining the effects of this peptide on evoked population spikes and EPSPs. In the CA1 region, the peak amplitude of population spikes induced by the stimulation of Schaffer collaterals was markedly depressed in the presence of 1 μM orphanin FQ (Fig. 1A). The effect had an onset within 3–4 min and reached the plateau in 10 min following perfusion. The inhibition could be reversed by perfusing drug-free CSF for 10–15 min. Likewise, orphanin FQ (1 μM) caused a 44 ± 7% (n = 5) reduction in the EPSP slope relative to the pre-drug level



($P < 0.001$) but did not cause any significant change in the size of the fiber volley (data not shown). Figure 1B shows the input-output curve constructed by varying the stimulus intensity and plotting the EPSP slope as a function of the peak-to-peak amplitude of the fiber volley, indicating that orphanin FQ decreased EPSP slope at a given afferent input strength. Similar results were obtained in five experiments.

Orphanin FQ also inhibited population spikes recorded in the dentate gyrus. Following bath application of 1 μM orphanin FQ, the amplitude of granule cell population spike was attenuated by 16 ± 2% (n = 6). Compared to the CA1 region (30 ± 3% of reduction, n = 11), orphanin FQ appears to have less inhibitory effect on synaptic transmission in the dentate gyrus ($P < 0.005$, unpaired Student's *t*-test) (Fig. 1C).

We further examined the concentration-response relationship of the orphanin FQ effect in the CA1 region. The peptide was applied to three groups of slices for 10 min at 0.1, 1, and 10 μM concentrations. As shown in Figure 1D, orphanin FQ reduced the peak amplitude of population spike by 12 ± 1% (n = 5), 30 ± 3% (n = 11), and 55 ± 11% (n = 5), respectively. The inhibition caused by 10 μM orphanin FQ was significantly higher than that resulting from lower concentrations (0.1 and 1 μM, $P < 0.005$ and $P < 0.01$, respectively), demonstrating that the inhibitory effect was dose dependent.

An Inactive Analog of Orphanin FQ Does Not Inhibit Synaptic Transmission

The N-terminal tyrosine residue of opioid peptides is critical for binding to opioid receptors (Walker, 1982; Aloyo, 1992; Di Giannuario et al., 1993). We have synthesized the des-Phe[1] analog of orphanin FQ by eliminating the N-terminal phenylalanine

FIGURE 1. Orphanin FQ inhibits synaptic transmission in rat hippocampus. A: Orphanin FQ depresses somatic field potentials in hippocampal CA1 region. The dot plot shows the time course of synaptic depression following orphanin FQ application in CA1. Population spikes evoked by stimulation of Schaffer collaterals in artificial CSF were collected as baseline responses (a). Application of 1 μM orphanin FQ reduced the population spike amplitude by 40% (b). This effect could be washed out completely within 10 min of artificial CSF perfusion (c). The upper panel represents field potentials prior to (a) and during (b) orphanin FQ application and after (c) washout with artificial CSF. The duration of orphanin FQ application is indicated by a solid bar. B: Orphanin FQ inhibits dendritic field potentials in the CA1 region. The input-output curve was constructed by varying the stimulus intensity (0.08 ~ 0.25 mA) and plotting the EPSP slope as a function of the peak-to-peak amplitude of the fiber volley. Dotted and solid lines were polynomial fits to predrug and postdrug data, respectively. C: Bath application of orphanin FQ attenuates synaptic transmission in dentate gyrus (DG) and CA1 regions. In the presence of 1 μM orphanin FQ, the peak amplitude of population spikes was significantly reduced in both DG (84 ± 2% of the baseline, n = 6, $P < 0.001$) and CA1 regions (69 ± 3% of the baseline, n = 11, $P < 0.001$). The asterisk indicates $P < 0.005$. D: The inhibitory effect of orphanin FQ on synaptic transmission is dose dependent. In CA1 region, bath application of orphanin FQ at 0.1, 1, and 10 μM concentrations reduced the peak amplitude of population spikes by 12 ± 1% (n = 5), 30 ± 3% (n = 11), and 55 ± 11% (n = 5), respectively.

residue and examined its activity in receptor binding as well as its effect on hippocampal synaptic transmission. Des-Phe[1]-orphanin FQ receptor binding was examined in a competitive assay using P2 membrane preparation from rat brain. As demonstrated in Figure 2A, orphanin FQ exhibited a concentration-dependent displacement of ^{125}I-labeled orphanin FQ, with an apparent K_d value of approximately 6 nM. In contrast, des-Phe[1]-orphanin FQ caused negligible displacement even at a concentration as high as 10 μM, suggesting that this analog was inactive in the receptor binding assay.

In accordance with the result from the binding assays, bath application of 1 μM des-Phe[1]-orphanin FQ to hippocampal slices caused no significant change in both population spike amplitude and EPSP slope in the CA1 region ($P < 0.005$ for both, Fig. 2B). After washing the slices with artificial CSF, the subsequent perfusion of 1 μM orphanin FQ reduced population spike and EPSP slope by 24 ± 2% (n = 5) and 30 ± 1% (n = 4), respectively. This inhibitory effect could be washed out after a 20- to 30-min artificial CSF perfusion.

Orphanin FQ Increases Paired-Pulse Facilitation in CA1

Orphanin FQ-induced reduction in the EPSP slope indicates that the peptide may depress synaptic transmission by reducing presynaptic transmitter release. To further test this possibility, we examined the effect of orphanin FQ on paired-pulse facilitation. Paired-pulse facilitation is known to reflect a presynaptic enhancement in transmitter release (Zucker, 1989). Previous studies have shown that slices maintained under conditions which decreased transmitter release increased paired-pulse facilitation (Mallart and Martin, 1968; Katz and Miledi, 1968; Creager et al., 1980). Our results from seven slices showed that the inhibition of orphanin FQ is associated with a 11 ± 4% increase in facilitation ($P < 0.05$, paired Student's *t*-test). The increased paired-pulse facilitation suggests that activation of ORL_1 receptors by orphanin FQ may reduce transmitter release at synapses between Schaffer collaterals and CA1 pyramidal neurons.

To further test whether orphanin FQ might cause any change in postsynaptic excitability, we investigated the relation between EPSP slope and population spike at a range of stimulus intensities. Because the slope of the field EPSP is proportional to local inward currents underlying postsynaptic depolarization (Andersen et al., 1978), by looking at the change in population spike at given EPSP slope over a range of stimulus intensities, we can examine whether the depressant effect on synaptic transmission caused by orphanin FQ is due to reduced firing probability at a given excitatory postsynaptic current. In the presence of orphanin FQ, three out of

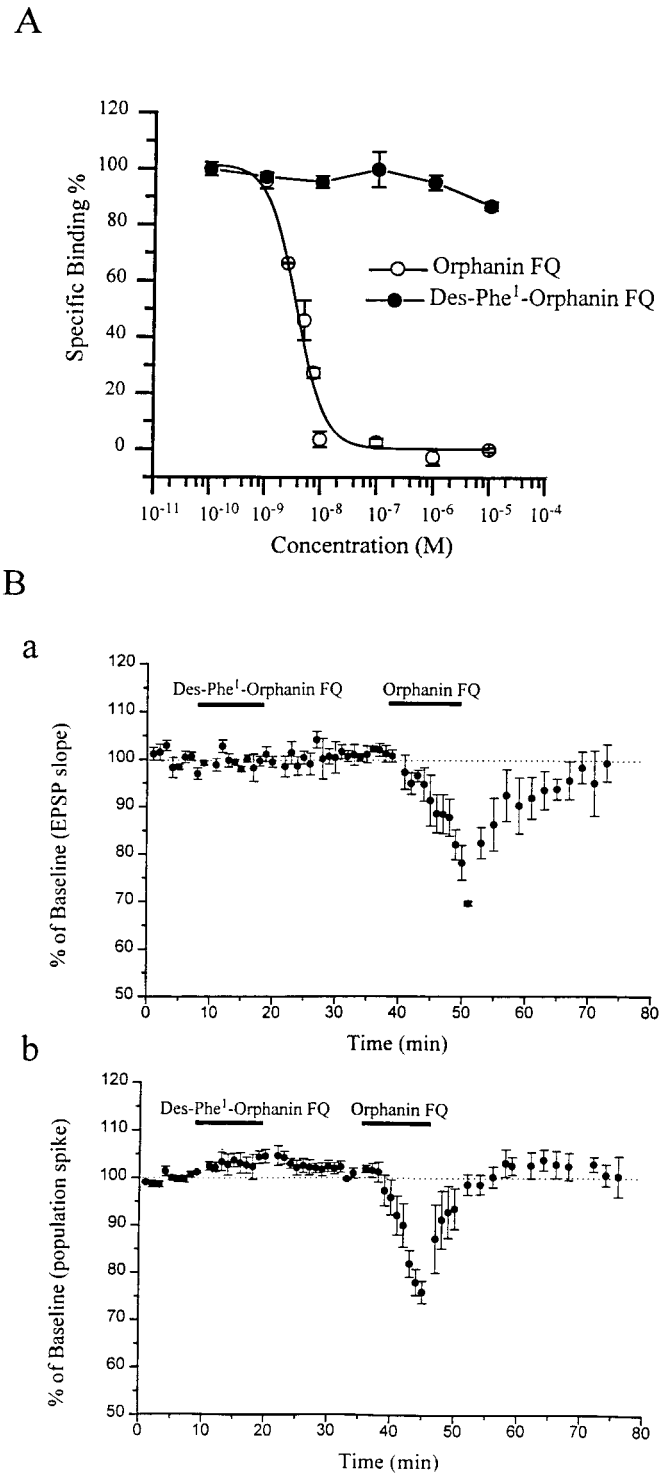


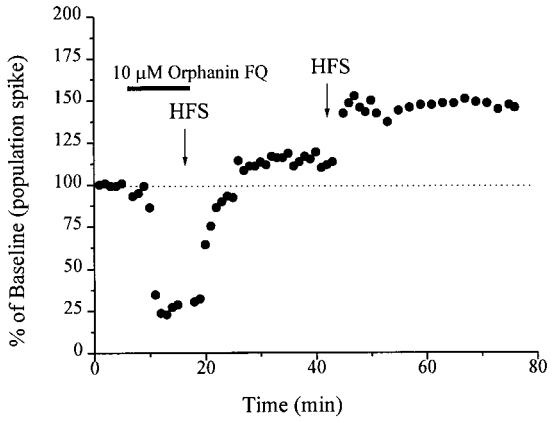
FIGURE 2. Des-Phe[1]-orphanin FQ does not bind to orphanin FQ binding sites and fails to inhibit synaptic transmission. A: Orphanin FQ, but not des-Phe[1]-orphanin FQ, displaces the binding of ^{125}I-labeled orphanin FQ. Competitive binding assays were performed by adding increasing concentration of unlabeled orphanin FQ (open circle) or des-Phe[1]-orphanin FQ (solid circle). Orphanin FQ caused displacement in a concentration-dependent manner, whereas des-Phe[1]-orphanin FQ showed only negligible displacement even at 10 μM. Each data point is the mean of triplicate determinations. The maximum specific binding in the absence of competitors was defined as 100%. Error bars represent the standard error of mean (SEM). B: Des-Phe[1]-orphanin FQ (1 μM) had no effect on both EPSP slope (a) and population spike amplitude (b) in the CA1 region. However, in the same slices, 1 μM orphanin FQ subsequently attenuated the amplitude of population spike and EPSP slope by 24 ± 2% (n = 5) and 30 [6ewd]) 1% (n = 4), respectively. Solid bars indicate the duration of peptide applications.

seven slices showed no shift of EPSP-spike curve; however, the EPSP-spike curves slightly shifting to the right (n = 2) and to the left (n = 2) of the predrug curve were also observed. These results do not indicate a significant change in postsynaptic excitability caused by orphanin FQ.

Orphanin FQ Inhibits LTP in CA1

In CA1 region, high-frequency stimulation (4 pulses at 100 Hz, repeated every 200 ms for a total of 40 pulses) could produce robust LTP of both population spike amplitude and EPSP slope. In the presence of 10 μM orphanin FQ, LTP induction was essentially abolished (Fig. 3A). This inhibitory effect was reversible, because the second high-frequency stimulation, applied to the same slice after washout of orphanin FQ, could produce substantial LTP. A lower concentration of orphanin FQ (i.e., 1 μM) was also found to attenuate the induction of LTP (Fig. 3B). The increase in EPSP slope (12 ± 4%, n = 5) and population spike (20 ± 5%, n = 11), induced by high-frequency stimulation in orphanin FQ-treated slices, was significantly smaller than those in the control slices (43 ± 7%, n = 6 and 45 ± 5%, n = 12, respectively, $P < 0.005$ for both comparisons).

A



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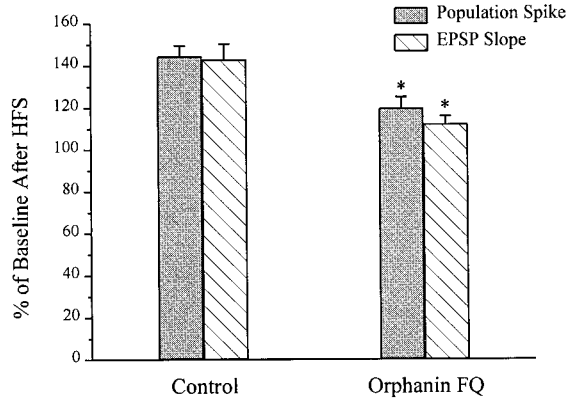

FIGURE 3. Ophanin FQ inhibits LTP in hippocampal CA1 region. A: In CA1, LTP induced by high-frequency stimulation (HFS, 4 pulses at 100 Hz, repeated every 200 ms for a total of 40 pulses) of Schaffer collaterals is blocked by 10 μM orphanin FQ. A stimulus intensity that evoked 50% of maximum response in artificial CSF prior to the HFS was chosen as a test pulse. The test pulses were applied at 0.02 Hz throughout the experiment for collection of both baseline and post-tetanus responses. Addition of orphanin FQ (10 μM) into the perfusion medium resulted in a 75% decrease in population spike amplitude in this slice. The first HFS (with an adjusted stimulus that induced the same amplitude as the baseline response) was then applied in the presence of orphanin FQ. Orphanin FQ was washed off immediately following the tetanus, and test pulses were resumed to collect post-tetanus responses for 30 min. As shown here, after the recovery from the peptide effect, less than 20% of increase in evoked responses was observed. After complete washout of orphanin FQ, a second high-frequency stimulation induced robust LTP (46% of potentiation). The duration of orphanin FQ application was indicated by a solid bar. B: This histogram summarizes the depressant effect of orphanin FQ on CA1 LTP. All the experiments were performed using the same paradigms described in A. HFS-induced potentiation of population spike amplitude (gray bar) and EPSP slope (hatched bar) were determined 30 min after high-frequency stimulation, relative to the pretrain baseline response. In the control group, LTP of population spike amplitude (45 ± 5%, n = 12) and EPSP slope (43 ± 7%, n = 6) were obtained by applying high-frequency stimulation to Schaffer collaterals in artificial CSF. In the presence of 1 μM orphanin FQ, high-frequency stimulation-induced enhancement of population spike (20 ± 5%, n = 11) and EPSP slope (12 ± 4%, n = 5) was significantly reduced, as compared to the control. Asterisks indicate $P < 0.005$.

DISCUSSION

Orphanin FQ Can Function as an Inhibitory Modulator in Hippocampus

Orphanin FQ can modulate neurotransmission and synaptic plasticity in both the CA1 and dentate gyrus of the rat hippocampus. The strong inhibitory effect on the EPSP slope and population spike amplitude suggests that orphanin FQ can act as an inhibitory modulator and may play an important role in regulating hippocampal synaptic transmission. The orphanin FQ effect is similar to that reported for the κ receptor agonist dynorphin A (Wagner et al., 1993; Weisskopf et al., 1993; Drake et al., 1994) but opposite to opioid agonists for μ and δ receptors (Bostock et al., 1984; Piguet and North, 1993; Watson and Lanthorn, 1993). The orphanin FQ-induced inhibition is concentration dependent and presumably caused by activation of ORL_1 receptors. Three lines of evidence support the receptor specificity of orphanin FQ effects. First, our receptor binding assays confirm that orphanin FQ displays specific binding. Second, des-Phe^1-orphanin FQ that was inactive in the binding assay failed to inhibit synaptic transmission. Third, in accordance with our

previous observation of a higher ORL_1 receptor density in CA1 than in DG (Anton et al., 1996), the percentage of inhibition produced by orphanin FQ in CA1 was larger than that in the dentate gyrus.

Our results showed that orphanin FQ reduced EPSP slope but significantly increased paired-pulse facilitation of EPSP in CA1, suggesting that the inhibitory effect of this peptide, like the dynorphin effect (Weisskopf et al., 1993), may involve a presynaptic modification on synaptic transmission. One possible mechanism is that orphanin FQ depresses synaptic transmission by decreasing presynaptic release of excitatory transmitter. ORL_1 receptors may be present on Schaffer collateral terminals, and their activation reduces presynaptic transmitter release. In fact, the presence of abundant ORL_1 receptors in the cell body layer (stratum pyramidale) and the fiber processes (stratum radiatum) of the CA1 region has been demonstrated (Anton et al., 1996). Nonetheless, the cellular localization of the ORL_1 receptor on CA1 pyramidal neurons and their synaptic inputs is still not clear. Further anatomical and neurochemical studies are necessary to elucidate the cellular mechanism of the peptide effect.

The N-Terminal Phenylalanine Residue of Orphanin FQ Is Critical for Receptor Binding and Inhibition of Synaptic Transmission in the Hippocampus

One of the common motifs among the endogenous opioid peptides is the N-terminal sequence Y-G-G-F. Previous studies have demonstrated that eliminating the N-terminal amino acid of opioid peptides resulted in the loss of receptor binding and biological activity (Walker, 1982; Aloyo, 1992; Di Giannuario et al., 1993). One such example is des-Tyr^1-dynorphin (2–17) without the N-terminal tyrosine, which no longer binds to opioid receptors (Walker, 1982). Although des-Tyr^1-dynorphin has no opioid effect, nonopioid actions of this peptide have been reported (Walker, 1982; Moore et al., 1994; Schwyzer, 1995; Shen and Crain, 1995; Claye et al., 1996). To determine whether orphanin FQ has analogous structural activity relationships, we eliminated the first amino acid, phenylalanine, from the N terminus of orphanin FQ. The results showed that des-Phe^1-orphanin FQ failed to compete with iodinated orphanin FQ in receptor binding assays and had no depressant effect on synaptic transmission. These results demonstrate that the first amino acid at the N terminus is critical for orphanin FQ to bind to its receptors and confirm the specificity of the inhibitory effect of orphanin FQ.

Orphanin FQ Modulates LTP in CA1

Because synaptic transmission in CA1 was strongly modulated by orphanin FQ, it is of interest to determine whether activation of the ORL_1 receptor would also affect synaptic plasticity such as LTP. Our results demonstrate that high-frequency stimulation of Schaffer collaterals induced significantly smaller potentiation of evoked responses in orphanin FQ-treated slices, as compared with the control group. Because the stimulus for inducing LTP in the presence of orphanin FQ was adjusted to evoke the same amplitude of the baseline response and the potentiation in both groups was measured 30 min after high-frequency stimulation in the absence of orphanin FQ, the suppression on LTP induction during high-frequency stimulation, rather than a direct inhibition on synaptic transmission by the peptide, should be responsible for the attenuated enhancement in the orphanin FQ-treated slices.

All three major types of classical opioid receptors have been previously shown to modulate LTP induction in the hippocampus. Opioids acting on μ or δ receptors reportedly facilitate induction of LTP (Bramham et al., 1991; Xie and Lewis, 1991; 1995b), whereas dynorphin inhibits LTP through κ receptors (Wagner et al., 1993; Weisskopf et al., 1993). Our results indicate that the effect of orphanin FQ on hippocampal LTP is similar to dynorphin but opposite to μ and δ opioids, suggesting that ORL_1 receptor activation in the hippocampus can modulate use-dependent synaptic plasticity involved in learning and memory. The counterbalance between the ORL_1 receptors and μ/δ opioid receptors may play an important role in regulating the synaptic efficacy and functional plasticity.

In contrast with the facilitation of μ and δ opioids on locomotion and dopamine release (Olson et al., 1994), orphanin FQ also exhibits inhibitory effects (Reinscheid et al., 1995; Murphy et al., 1996). Nevertheless, the cellular mechanisms underlying the different effects of orphanin FQ and opioids are likely complicated because orphanin FQ as well as the classical opioids both inhibit adenylate cyclase and reduce the intracellular level of adenosine 3′,5′-monophosphate (cAMP) (Meunier et al., 1995; Reinscheid et al., 1995). Our findings and other previous studies have suggested that activation of the ORL_1 receptor by orphanin FQ may functionally antagonize μ and δ opioid effects in different brain areas and pathways. Further studies examining the possibility of coexistence of orphanin FQ and μ/δ opioids as well as colocalization of the ORL_1 receptors with the μ/δ receptors will be necessary to explore the possible mechanism of counterbalance between these peptide activities involved in the physiological function at the network or system level.

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