Effects of orphanin FQ on central dopaminergic neuronal activities and prolactin secretion

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Received 24 February 2000; accepted in final form 9 October 2000

Shieh, Kun-Ruey, and Jenn-Tser Pan. Effects of orphanin FQ on central dopaminergic neuronal activity and prolactin secretion. Am J Physiol Regulatory Integrative Comp Physiol 280: R705–R712, 2001.—Effects of orphanin FQ (OFQ) on central dopaminergic (DA) neurons and serum prolactin (PRL) were examined in ovariectomized, estrogen-primed Sprague-Dawley rats. The activities of central DA neurons, including the tuberoinfundibular (TI), nigrostriatal, mesolimbic, and incertohypothalamic ones, were determined by measuring the levels of 3,4-dihydroxyphenylacetic acid (DOPAC), the major metabolite of dopamine, in their projection regions in the brain by HPLC plus electrochemical detection. Intracerebroventricular administration of OFQ lowered DOPAC levels in the median eminence (ME), striatum, nucleus accumbens, and hypothalamic paraventricular nucleus in a dose (0.01–10 μg)- and time (30–90 min)-dependent manner. In contrast, OFQ increased DOPAC in the suprachiasmatic nucleus and had no effect in the periventricular nucleus. Serum PRL levels exhibited a typical inverse relationship with the activity of TIDA neurons, as determined by DOPAC levels in the ME. In the afternoon, we observed an endogenous decrease of ME DOPAC level accompanied by a PRL surge in estrogen-primed female rats. Although OFQ caused further decrease of ME DOPAC in the afternoon, it failed to augment the PRL surge level. Although pretreatment of an antisense oligodeoxynucleotide against the opioid receptor-like receptor gene had no effect on basal ME DOPAC levels in the morning or afternoon, it attenuated the afternoon PRL surge. Furthermore, it blocked the effects of exogenous OFQ on ME DOPAC and serum PRL levels, whereas the sense or missense oligodeoxynucleotide had no effect. These results indicate that OFQ and its receptors may be involved in the regulation of central DA neuronal activity and PRL secretion.
	nociceptin; tuberoinfundibular dopaminergic neuron; opioid receptor-like; antisense oligodeoxynucleotide

Orphanin FQ (OFQ) or nociceptin is a recently cloned heptadecapeptide that belongs to the opioid peptide family (24, 36). It exhibits nanomolar binding affinity to the previously cloned opioid receptor-like (ORL1) orphan receptor (25) and is regarded as the endogenous ligand for that receptor. Both OFQ and its receptor are widely distributed in the central nervous system with exceptionally high density in the hypothalamic and midbrain regions (30, 31).

OFQ has been shown to activate an inward rectifying K+ channel in neurons of hypothalamic arcuate nucleus that express tyrosine hydroxylase or endorphin (44). OFQ can also stimulate PRL secretion in both male and female rats (6). Whether the latter effect is acting via inhibiting the TIDA neurons has not been reported except for two preliminary studies (5, 41) and was the objective of this study. In addition, we (20, 39) previously showed that both TIDA neuronal activity and PRL secretion exhibit diurnal changes in female rats, and endogenous opioids may play a significant role (40); thus OFQ was determined to have both morning and afternoon effects. Since there was no specific OFQ antagonist available, we used instead an antisense oligodeoxynucleotide (ODN) against the exon 3 of ORL1 receptor gene to verify the presumed effects of OFQ.

In addition to TIDA neurons, we also examined the activities of nigrostriatal (NS), mesolimbic (ML), and incertohypothalamic (IH) DA systems by measuring the dopamine metabolite levels in their terminal regions, i.e., striatum (ST) for NSDA, nucleus accumbens (NA) for MLDA, hypothalamic paraventricular (PVN), suprachiasmatic (SCN), and periventricular nuclei for the IHDA system. The effects of opioids on NSDA, MLDA, and IHDA systems are known to be different from those of the TIDA system, namely, stimulatory instead of inhibitory (2, 16, 42). It is then of interest to know if OFQ exhibits similar or dissimilar effects in these systems.

MATERIALS AND METHODS

Animals and experimental procedures. Adult female Sprague-Dawley rats, weighing 220–250 g, were purchased...
from Yang-Ming University Animal Center (Taipei, Taiwan). All animals were housed in a temperature (23±1°C) and light (lights off from 0600 to 2000)-controlled room with free access to rat chow and tap water. All rats were ovariectomized (OVX) and, 1 wk later, implanted with silicone capsules (ID, 1.57 mm; OD, 3.18 mm; active length, 20 mm; A-M Systems, Everett, WA) containing 17β-estradiol (E2; Sigma, St. Louis, MO; 150 μg/ml corn oil; Sigma) for another week. One week before experiment, using a stereotaxic instrument we implanted each rat with a cannula (23-gauge stainless steel tubing) in its right lateral cerebroventricle. Ether and Equithesin were used as anesthetics for ovariectomy and stereotaxic surgery, respectively.

All rats received drug injections through the preimplanted cannulas around either 0900 in the morning or 1400 in the afternoon. The rats were then quickly decapitated at a specific time point after the injection. Their brains were removed and frozen on dry ice, and their serum samples were collected and stored at −20°C until assayed for PRL levels. The frozen brain was cryosectioned on the same day with a tabletop freezing microtome. Thick (600 μm) coronal brain sections were prepared and thaw mounted onto glass slides. The ME, ST, NA, PVN, SCN, and periventricular nuclei of each rat were dissected out from the sections by the micropunch technique (34). They were individually stored frozen in 40 μl of 0.15 M sodium phosphate buffer containing 0.65 mM sodium octanesulfonate, 0.5 mM EDTA, and 12% methanol, at pH 2.8, until assayed.

**Experimental design.** In the first experiment, 30 OVX+E2 rats were randomly divided into five groups. Each group received injection of either vehicle [artificial cerebrospinal fluid (aCSF)] or one of the four doses of OFQ (0.01, 0.1, 1, or 10 μg/3 μl icv; RBI, Natick, MA) in the morning around 0900, and each rat was decapitated 60 min after receiving the injection.

In the second experiment, 50 OVX+E2 rats were divided into four groups. Half of each group received injections of vehicle (aCSF) or OFQ (1 μg/3 μl icv) in the morning and half in the afternoon. The rats were decapitated at 30, 60, or 90 min after the injection.

In the third experiment, 75 OVX+E2 rats were divided into four groups. Each group received injections of vehicle (aCSF), the antisense, sense, or missense ODNs against exon 3 of the ORL1 receptor gene on days 1, 3, and 5 (10 μg/3 μl icv). The sequences of antisense, sense, and missense ODNs are 5′-GGG CTG TGC AGA AGC CGA GA-3′, 5′-TCT CGG CTT CTT CAC AGC CC-3′ and 5′-GGG TCG GTC AGA GAC CGA GA-3′, respectively. The sequences were adapted from a previous study (15) and were synthesized by a local company (DNAFax, Taipei, Taiwan). On day 6, each group was further divided into three: two received injections of aCSF in the morning or afternoon, and the third received OFQ (1 μg/3 μl
icv) in the morning. All rats were decapitated 60 min after the injection.

Assay and statistical analysis. The activity of central DA neurons was assessed by measuring the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), a major metabolite of dopamine, using HPLC coupled with electrochemical detection (ECD) as reported previously (20, 39, 40). In brief, brain samples were thawed, sonicated, and centrifuged. The supernatant was injected into an HPLC-ECD system (Bioanalytical Systems LC480, with PM-80 pump, Rheodyne 7125 injector, phase II octadecylsilane column, 3.2×100 mm with 3-μm sphere and LC-4C electrochemical detector; Bioanalytical Systems, West Lafayette, IN). The mobile phase was identical with the tissue buffer used in storing the punched brain tissues. The flow rate of the pump was 0.8 ml/min, and the oxidizing potential was set at +0.75 V. The tissue pellets were dissolved in 1.0 N NaOH and assayed for their protein contents (17). Data were expressed as nanograms DOPAC per milligram protein.

The materials used for rat PRL radioimmunoassay were kindly provided by Dr. A. F. Parlow at the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases. The iodinated PRL used was rat PRL I-6, the PRL standard was rat PRL RP-3, and the antibody was antirat PRL S-7. The intra- and interassay coefficients of variance were 5 and 7%, respectively (n = 20).

One-way ANOVA followed by Student-Newman-Keuls' multiple-range test was used to test the significance of difference among groups. A P < 0.05 was considered as significant in difference.

RESULTS

Dose-dependent effects of OFQ. Intracerebroventricular injection of OFQ in various doses (0.01–10 μg) exhibited dose-dependent inhibitory and stimulatory effects on ME DOPAC and serum PRL levels, respectively, 60 min after the injection (P < 0.01; Fig. 1). ME DOPAC seemed to respond more sensitively to OFQ than did serum PRL, in that 0.1 μg OFQ was effective for the former, whereas doses 1 μg and larger were needed for the latter (Fig. 1).

OFQ at doses 0.1 μg or higher also significantly inhibited DOPAC concentrations in the ST, NA, and PVN (P < 0.05 and 0.01; Fig. 2), whereas it stimulated and had no effect on those in the SCN (P < 0.01) and periventricular nuclei, respectively (Fig. 2).

Time-dependent effect of OFQ. Diurnal changes of ME DOPAC and serum PRL levels in OVX+E2 female rats were observed as previously reported (20, 39, 40). OFQ (1 μg) given in the morning significantly lowered ME DOPAC levels 60 and 90 min later (P < 0.01; Fig. 3, top left) and stimulated serum PRL at 60 min (P < 0.01; Fig. 3, bottom left). Similar injection in the afternoon further lowered the already low ME DOPAC levels 30–90 min after the injection (P < 0.01; Fig. 3, top right). Nevertheless, the afternoon surge levels of serum PRL were not significantly affected by OFQ (Fig. 3, bottom right).

No diurnal difference was observed in the DA activity of any other region (Fig. 4). OFQ lowered the DOPAC levels in the NA, ST, and PVN at 30–90 min and stimulated those in the SCN at 60 and 90 min, both in the morning and in the afternoon (P < 0.01; Fig. 3, bottom right).

Fig. 3. Time-dependent effect of OFQ on ME DOPAC and serum PRL levels of OVX + E2 rats. OFQ (1 μg icv) was given at either 0900 or 1400, and the rats were decapitated 30, 60, or 90 min later. The vertical line above each bar represents the SE (n = 6). *P < 0.05; **P < 0.01 compared with respective vehicle-treated control (open bars).

Fig. 4. No effect was found in the periventricular nucleus.

Effect of antisense ODN against ORL1 receptor. Pretreatments of three different ODNs from days 1 to 5 had no significant effect on basal DOPAC levels in the ME, ST, NA, PVN, or SCN on day 6 (Figs. 5 and 6). Serum PRL levels were not affected either (Fig. 5). Nevertheless, pretreatment of antisense ODN against exon 3 of the ORL1 gene blocked the inhibitory effects of OFQ on DOPAC levels in the ME, ST, NA, and PVN, and the stimulatory effect on the SCN (P < 0.01; Figs. 5 and 6). Similarly, the effect of OFQ on serum PRL levels was prevented by the antisense ODN. Similar treatments of sense or missense ODN were without effect for the action of OFQ (Figs. 5 and 6). Again, no change was found in the periventricular nucleus by these treatments (data not shown).

As for the diurnal changes of ME DOPAC and serum PRL levels, pretreatment of antisense ODN against exon 3 of ORL1 gene had no effect on the afternoon decrease of ME DOPAC levels but did significantly attenuate the afternoon PRL surge (P < 0.01; Fig. 7). Treatments of sense or missense ODN were not effective either.
DISCUSSION

Since its discovery in 1995, OFQ has been extensively studied, mostly on its role in pain and analgesia (8). A few studies demonstrate OFQ’s effect on central DA neurons (14, 28, 29), but only one details its effect on PRL secretion (6). Using OFQ and the antisense ODN against its receptor gene as tools, we obtained the following findings:

1) OFQ concomitantly inhibited TIDA neuronal activity and stimulated PRL secretion;
2) OFQ inhibited MLDA and NSDA neuronal activities;
3) OFQ exhibited differential effects on different IHDA neuronal activities, i.e., inhibition in the PVN, stimulation in the SCN, and no effect in the periventricular nucleus;
4) OFQ may exhibit its effect via specific ORL1 receptors; and
5) depletion of endogenous ORL1 receptor significantly affected the afternoon PRL surge, but not the activity of central DA neurons.

The possible effect of OFQ on TIDA neurons has been indicated but not proven (44). With the use of intracellular recording in brain slices, OFQ can have a hyperpolarizing effect on tyrosine hydroxylase-, β-endorphin-, and gonadotropin-releasing hormone-immunoreactive neurons in the arcuate nucleus. Apart from technical difficulty that limits the number of arcuate neurons one can record and identify, tyrosine hydroxylase-immunoreactive neurons in the arcuate nucleus can also be DOPAergic, in addition to DA neurons (23, 39). Using direct measurement of the major dopamine metabolite in the terminal region of TIDA neurons, we provide here stronger evidence supporting OFQ’s inhibitory effect on TIDA neurons.

This inhibitory effect also corresponds well with OFQ’s stimulatory effect on serum PRL levels. A recent study (6) reported that central administration of OFQ stimulates PRL secretion in both male and female rats, a finding that was confirmed in this study. Through concurrent determination of TIDA neuronal activity and serum PRL level in the same animal, we claim that OFQ exerts its stimulatory effect on PRL secretion via inhibiting the TIDA neurons. Both our dose- and time-dependent studies support this notion. The only inconsistent finding was observed when we gave OFQ in the afternoon. Because the afternoon ME DOPAC and serum PRL levels were already low and high, respectively, slight decreases in ME DOPAC levels might not cause further PRL secretion.

As for the role of endogenous OFQ, the picture is less clear. It is well established that endogenous opioids play an inhibitory role in the control of TIDA neurons.
and a stimulatory one on PRL secretion (26, 35). Both μ- and κ-receptors are involved (2, 21, 40). Although pretreatment of the antisense ODN blocked the effect of exogenous OFQ, it failed to exhibit a significant effect on basal levels and diurnal change of ME DOPAC. Thus endogenous OFQ may not exhibit a tonic control over basal TIDA neuronal activity, nor does it have a phasic control over the diurnal change of TIDA neurons. Nevertheless, treatment of the antisense ODN indeed attenuated the afternoon PRL surge, indicating that endogenous OFQ may still play a role in the control of PRL secretion.

The disparate findings between afternoon ME DOPAC and serum PRL levels in Figs. 3 and 7 may have several possible explanations. First, although exogenous OFQ could further lower the afternoon ME DOPAC level, removal of its endogenous action had no significant effect. This may indicate the dominance of other opioid systems at the time (40). Second, the PRL surge has been shown to involve both lowering the inhibition of dopamine and stimulation of a yet-to-be-identified PRL-releasing factor (39). The possibility that OFQ may affect the secretion of a PRL-releasing factor cannot be excluded. Third, the ME DOPAC is a compromising index for TIDA neuronal activity (26, 35), because only a portion of dopamine released is taken back by the presynaptic terminal and metabolized to DOPAC. (The rest is taken by portal vessels and sent to the anterior pituitary.) Thus an inverse relationship between ME DOPAC and serum PRL level can be observed in most, but not all, our experiments.

A recent preliminary report (5) using intracerebroventricular injection of OFQ (1 μg), however, did not find an effect on ME DOPA accumulation in male rats. The apparent contradiction with this study may be due to the different animal model used. The gender difference of TIDA neuronal activity, i.e., higher in female than in male, is well known (9). Part of the reason is that the male has a higher endogenous opioidergic tone, which constantly inhibits the TIDA neuron (22). The TIDA neurons of the male rat can be activated by nor-binal-torphimine, a κ-receptor antagonist, but have no response to U-50488, a κ-agonist; whereas the reverse is true for the female: inhibition by U-50488 and no response to nor-binal-torphimine.

As for the MLDA and NSDA systems, the consensus is that opioids increase their activities by lowering the activities of inhibitory GABAergic neurons (13). As for OFQ, the picture is not as clear yet. Both inhibitory (28) and stimulatory (12) effects of OFQ on locomotor activity have also been reported. OFQ applied to the cerebroventricle (28) or ventral tegmental area (29) has been shown to reduce dopamine release in the NA as sampled by microdialysis. On the other hand, OFQ perfused into the ST may enhance the release of dopamine (14). A recent report (32) showed that ORL1 receptor mRNA colocalizes with tyrosine hydroxylase-containing neurons in the ventral tegmental area and substantia nigra, indicating that OFQ may have a direct effect on NSDA and MLDA neurons. Using intracerebroventricular injection of OFQ, we found that it inhibited both NSDA and MLDA neuronal activities. Thus the overall effect of OFQ on NSDA and MLDA neurons should be inhibitory.

The IHDA neuronal system is a diverse one that comprises DA neurons in the medial zona incerta (A13) and rostral hypothalamic periventricular nucleus and their projections within and outside the hypothalamus (4, 11, 27). The functional role(s) played by the IHDA system has not been ascertained, although it may involve the neuroendocrine control of luteinizing hormone secretion (18, 19, 38), as well as sexual (10) and ingestive (43) behaviors in rats. Earlier studies (16, 42) indicate that IHDA neurons behave like the NSDA and MLDA neurons in their responses to opioids, i.e., being
stimulated. It is then of interest to find that OFQ exhibited differential effects on DA activities in the SCN, PVN, and periventricular nucleus, i.e., stimulation, inhibition, and no effect, respectively.

High density of ORL1 receptor mRNA has been shown to exist in both PVN and SCN (30), and OFQ has been shown to modulate neuronal activities in the SCN (1). Because few IHDA neurons are found in the PVN and SCN, OFQ may act through other neurons within the PVN and SCN to affect dopamine release and metabolism. However, direct action of OFQ on somas or terminals of IHDA neurons cannot be excluded. Determining exactly which PVN and SCN neuron(s) OFQ does affect and how that affects the IHDA neuronal activity awaits future study.

Because no ideal OFQ receptor antagonist was available (7), we used an antisense ODN against the exon 3 region of ORL1 receptor gene, with the hope of preventing the action of OFQ. Although exact changes of ORL1 receptor mRNA or binding activity after antisense ODN treatment were not determined, the effectiveness of the treatment in preventing the effects of OFQ in all the regions tested supports our hypothesis that OFQ indeed exerts its effect through a specific ORL1 receptor. The negative findings from using sense or missense ODN further strengthen the specificity of the antisense ODN used. The same antisense ODN has already been used in previous studies to prevent OFQ-induced analgesia (37) or hyperphagia (15).

In summary, exogenous OFQ exhibits potent effects on central DA systems and PRL secretion. The physiological role of endogenous OFQ, however, remains to be elucidated.

The authors are grateful for the technical assistance of Z. F. Yuan and S. C. Yang.

This study was supported in part by National Science Council of the Republic of China Grants NSC 89–2321-B010–038 and NSC 89–2320-B010–034 (to J.-T. Pan).

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Fig. 7. Effects of pretreatment of AS, S, or MS ODNs against exon 3 of ORL1 gene on morning and afternoon levels of ME DOPAC and serum PRL of OVX + E2 rats. The ODNs (10 μg/3 μl icv) were injected on days 1, 3, and 5. The rats were decapitated around either 1000 or 1500. The vertical line above each bar represents the SE (n = 6). ***P < 0.01 compared with morning level of respective pretreated group. **P < 0.01 compared with vehicle-pretreated control at the same time point.

REFERENCES


