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Estrogen-dependent, sex-specific modulation of mustard oil-induced secondary thermal hyperalgesia by orphanin FQ in the rat

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Abstract

Activation of opioid receptor-like 1 receptor (ORL_1) by intrathecal administration of orphanin FQ (OFQ), an endogenous ligand for the ORL_1 receptor, has been shown to produce antinociception. In addition, we have recently shown gonadal hormone-dependent, sex-specific modulation of acute spinal nociception such that estrogen attenuated OFQ-induced antinociception in the female whereas testosterone was required for the expression of antinociception in the male. However, sex-related differences in the role of OFQ under hyperalgesic conditions are unknown. Hence, we investigated whether OFQ produces sex-specific modulation of mustard oil-induced secondary thermal hyperalgesia in the rat. Mustard oil application to the hind limb significantly reduced the tail flick latencies (TFL) in male, and ovariectomized (OVX), estradiol treated ovariectomized (OVX+E), proestrous (ProE) and diestrous (DiE) females. Intrathecal administration of OFQ not only attenuated mustard oil-induced decrease in TFLs, i.e. reversed hyperalgesia, but also led to a significant increase in TFLs above the baseline, i.e. produced antinociception in male, OVX, and diestrous rats. However, OFQ failed to alter TFLs in proestrous or OVX+E females, thus these two groups with elevated estrogen levels remained hyperalgesic following mustard oil treatment. These findings demonstrate that OFQ modulates mustard oil-induced secondary hyperalgesia in an estrogen-dependent, sex-specific manner.

Keywords

Sex-differences; nociception; orphanin FQ; tail flick; mustard oil; spinal cord

Introduction

Activation of opioid receptor like-1 receptor (ORL_1) [31] using intrathecal (i.t.) application of its endogenous ligand, orphanin FQ (OFQ) [25] into the spinal cord has been shown to produce primarily antinociceptive effects [5,14,29,42–44]. In addition, studies on ORL_1 receptor knockout or preproorphanin knockout mice show that these mice display an increased sensitivity to tonic inflammatory pain [8], implicating this opioid receptor system as a modulator of inflammatory pain. Further, antihyperalgesic effectiveness of OFQ on primary hyperalgesia induced by complete Freund's adjuvant has also been shown [11,12]. We [10,

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32,38] and others [9,13,26,35] have demonstrated sex-related differences in the modulation of nociception by various G-protein coupled receptors (GPCR) such as opioid (μ and κ) and α_2 noradrenergic receptors in human and animal studies. Moreover, we recently demonstrated that OFQ produces sex-specific modulation of acute spinal nociception, such that estrogen attenuates antinociception in the female whereas testosterone is required for the expression of antinociception in the male [5]. One of the major contributors of clinical pain is hyperalgesia associated with chronic or neuropathic pain states [37], however, there is a lack of information on sex-related differences in the antinociceptive effectiveness of OFQ utilizing models of pain which induce hyperalgesia. Therefore, we investigated whether i.t. administration of OFQ leads to estrogen-dependent, sex-specific modulation of secondary thermal hyperalgesia. We utilized a mustard oil (allyl isothiocyanate)-induced model of hyperalgesia [20,41] and investigated the effects of OFQ on the noxious heat-evoked tail-flick reflex test [7] prior to and following mustard oil application on the hind limb.

Materials and Methods

Animals

Male, female, and ovariectomized (OVX) female Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were housed in the animal care facility at Meharry Medical College certified by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) under a 12-hour light/dark cycle (light 7 am – 7 pm). Food and water were freely available. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Meharry Medical College and abided by the established guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals and International Association for the Study of Pain (IASP). Estrous cycle stages were determined by the standard vaginal smear method. Female rats were operated in the diestrous phase and underwent nociceptive testing at proestrous (high estrogen level) and diestrous (low estrogen level) after having established two successive regular estrous cycles.

Implantation of Intrathecal Cannulae

Cannulae were implanted in all animals as described before [38] under ketamine and xylazine anesthesia (72 and 4 mg/kg respectively; i.p.). Briefly, rat's head was secured in a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA) and an incision was made above the atlantooccipital membrane which was cleared to expose the dura. A stretched PE-10 cannula (Intramedic, Clay Adams, Parsippany, NJ, USA; dead volume of 10 μl) was inserted into the subarachnoid space through a small incision in the dura. The cannula was gently pushed caudally to a length of 8.5 cm to reach the lumbosacral enlargement and was secured with dental cement to the base of the skull. The wound was sutured and the animal was placed on a heating blanket until it regained consciousness. Animals were allowed to recover for 5–7 days before undergoing nociceptive testing. The position of the cannula was confirmed, at the end of testing, by administering 15 μl of 2% lidocaine which temporarily paralyzed the animal's hind limbs. Animals showing any signs of neurological impairment after surgery were sacrificed immediately.

Estrogen replacement

Ovariectomized (OVX) rats were given a 2 week recovery period prior to surgical implantation of cannulae. A single dose of estradiol benzoate (100 μg/100 μl of sesame oil) was administered subcutaneously (s.c.) 48 hours prior to testing. This regimen has been used previously in both neuroendocrinological [3,34] and nociceptive studies [5,19,32,38]. The rationale for using a 100 μg dose of estradiol was its ability to most reliably induce lordosis behavior in rats [6]. In addition, we have previously reported dose dependent effects of estrogen on OFQ-induced antinociception using acute nociceptive testing [5]. Control animal groups (OVX) received

vehicle injections (sesame oil, 100 μl; s.c.). We have previously measured serum estradiol concentrations in OVX rats treated with varying doses of estradiol and at different time points after injection [32], and in normally cycling females at proestrous and diestrous stages [5]. The estrogen levels were found to be within the physiological range 48 hours after estradiol injection, the time point when all nociceptive testing is conducted in the present study. Further, estradiol levels 48 hours after a 100 μg dose of estrogen were similar to that reported elsewhere [4] in normally cycling females at the proestrous stage [32]. Estradiol levels measured at the proestrous or the diestrous stage correlated well with the estrous cycle stage as determined by vaginal cytology [5].

OFQ

OFQ was administered intrathecally and the dose (10 nmol/10 μl) was selected from a detailed dose response study with acute nociceptive testing reported by us before [5]. There were no apparent sedative effects in animals given this dose of OFQ.

Tail-flick test

Tail flick latency (TFL) was measured automatically by a tail-flick analgesia meter (Model 33T, IITC Life Science, Woodland Hills, CA) as described before [5]. Heat stimulus was applied 3–7 cm from the tip of the rat's tail at three separate spots to prevent sensitization as a result of heating the same spot in succession. TFL was recorded every 2 min, therefore the interval between two successive stimulations at the same spot was 6 min. An automatic cut off latency of 15 sec. was set to prevent tissue damage. Three baseline readings (6–8 sec) were taken at 2 min intervals prior to mustard oil application. A change in the tail temperature, whether induced by alteration in hormones [18,33] or by fluctuation in room temperature and other factors, could be a confounding factor in the tail flick test [17]. To avoid this, a trigger temperature setting was used to automatically pre-warm the tail to 32°C in all groups. Each animal was used for only one treatment condition.

Mustard oil – induced model of secondary hyperalgesia

As described previously [20,41], the lateral surface of the right hind limb (~ 30 mm above the ankle) was shaved and the rats were allowed to acclimate for 15 min in a Plexiglas cylinder. Upon determination of 3 pre-drug baseline tail flick latencies and 5 min after intrathecal OFQ (10 nmol/10 μl) or vehicle injection, mustard oil [100%, 20 μl in male, OVX, estradiol-treated OVX (OVX+E), and diestrous (DiE) animals; 100%, 10 μl in proestrous (ProE) females] was applied topically to the shaved area and the animal was left undisturbed for a period of 5 min after which the tail-flick test was performed. As initial tail flick trials done with 20 μl of mustard oil in proestrous females produced behavioral responses that included abdominal writhing and escape attempts followed by a complete inhibition of the tail flick response (data not shown), a smaller volume of mustard oil (10 μl) was utilized in proestrous females which produced a comparable facilitation of the tail flick reflex. All chemicals were obtained from Sigma, St. Louis, MO, USA.

Data analysis

Data were analyzed using SPSS (SPSS Inc., Chicago, IL, USA). A repeated measures ANOVA with between- (Group, Drug) and within- (Time) group factors, and dependent variable (TFL) was conducted. Area under the curve (AUC) was calculated by trapezoid method using Prism (Graphpad Software, Inc., San Diego, CA, USA) for time course plots to obtain a single measure of the overall response and was analyzed by one way ANOVA. A post-hoc (Bonferroni) test was performed where ANOVA yielded a main effect. A *P*-value of <0.05 was considered significant. Data were plotted as Mean±SEM.

Results

Mustard oil induces secondary hyperalgesia in male and OVX females

Baseline TFLs were comparable among male, OVX and OVX+E animals (fig. 1A) indicating that basal pain perception levels did not differ between different groups. Topical application of mustard oil in male, OVX and OVX+E animals produced robust secondary thermal hyperalgesia evident by a significant reduction in TFLs as compared to baseline TFLs (Fig. 1A).

OFQ produces estrogen-dependent modulation of mustard oil-induced secondary thermal hyperalgesia

Intrathecal administration of OFQ, 5 minutes prior to the application of topical mustard oil, not only reversed mustard oil-induced decrease in TFLs, it produced significant increases in the TFL compared to baselines in male and OVX groups (Fig. 1A) thereby preventing mustard oil induced secondary hyperalgesia as well as producing antinociception. The effect of OFQ peaked within 5 minutes and lasted for ~30 minutes before the TFLs returned to baseline levels. Moreover, even after 30 min following OFQ injection, the TFLs in male and OVX groups remained above decreased TFLs observed in hyperalgesic control animals (mustard oil only); indicating an inhibition of mustard oil-induced secondary thermal hyperalgesia by OFQ in male and OVX animals. However, OFQ in the OVX+E group failed to modulate mustard oil-induced secondary thermal hyperalgesia and the TFLs remained below baseline levels, similar to those in mustard oil control groups (Fig. 1A). A cumulative analysis of OFQ's effectiveness as an antinociceptive agent was analyzed by measuring the area under the curve (Fig. 1B) which shows that OFQ is substantially effective in male and OVX animals against mustard oil-induced secondary thermal hyperalgesia.

Endogenous estrogen regulates OFQ-modulation of mustard oil-induced secondary thermal hyperalgesia

Having established an estrogen-dependent component of OFQ's modulation of mustard oil-induced secondary thermal hyperalgesia, it was of significance to determine whether such estrogen-dependent effects are pertinent to the fluctuating levels of estrogen that occur during the estrous cycle in normally cycling females. The antihyperalgesic effects of OFQ were assessed in normally cycling females during estrous phases of high (proestrous) and low (diestrous) levels of circulating estrogen (Fig. 2A). Baseline TFLs were comparable between pro- and diestrous animals indicating that basal pain perception levels did not differ between the two groups (Fig. 2A). Topical application of mustard oil (20 μl and 10 μl in diestrous and proestrous groups respectively) produced a robust secondary hyperalgesia evident by a significant reduction in TFLs (Fig. 2A) and similar to male and OVX groups represented in fig. 1A. As indicated in methods, a smaller volume of mustard oil (10 μl) was utilized in proestrous females due to the exaggerated behavioral responses observed with 20 μl of mustard oil and to achieve TFLs comparable to other groups. OFQ produced a significant increase in TFLs in mustard oil treated DiE animals (Fig 2A). Thus, its effect of reversing mustard oil-induced secondary thermal hyperalgesia is similar to that observed in the male and OVX groups (Fig. 1A). In contrast, OFQ did not increase TFL in ProE females. Area under the curve analysis demonstrates that OFQ is significantly more effective in DiE as compared to ProE females (Fig. 2B). These findings substantiate that fluctuating levels of estrogen in intact female animals regulate the antinociceptive effects of OFQ in response to mustard oil-induced secondary hyperalgesia.

Discussion

The present study is the first to demonstrate estrogen-dependent, sex-related modulation of mustard oil-induced secondary thermal hyperalgesia by OFQ. In the presence of endogenous or exogenous estrogen, OFQ failed to modulate secondary thermal hyperalgesia produced by mustard oil in OVX+E or proestrous (high estrogen) females whereas it produced antihyperalgesic and antinociceptive effects in the male, OVX and diestrous (low estrogen) animals. These findings expand upon our previous observations that OFQ produces sex-specific modulation of acute spinal and trigeminal nociception, producing antinociception in male, DiE and OVX females but not in cycling females at proestrous (high circulating estrogen) or estradiol treated OVX females [5,10]. Assessing the ability of OFQ to modulate acute versus mustard oil-induced nociception advances previous evidence by demonstrating the potential effectiveness of OFQ as an antinociceptive agent in conditions resembling clinical pain states.

Mustard oil has been demonstrated to reliably produce secondary thermal hyperalgesia that subsides in an hour or two [20,41]. It activates a subset of TRPV1 peptidergic unmyelinated afferents that also express TRPA1 channel [2] which in turn triggers descending facilitation [41]. Hyperalgesia comprises of primary and secondary elements which stem from different mechanisms. Primary hyperalgesia, occurring at the site of injury, results from peripheral nociceptor sensitization, while secondary hyperalgesia, occurring in the surrounding unaffected area, is mediated through central plastic changes in the dorsal horn [21,39,40]. Experimentation in humans has also demonstrated similar hyperalgesic effects of mustard oil [22,35].

Our study revealed that the magnitude of secondary thermal hyperalgesia induced by mustard oil is similar among male, OVX, OVX+E, and DiE animals; mustard oil alone significantly facilitated tail flick latencies in these groups. Interestingly, proestrous females required only half the dose of mustard oil as compared to other groups (10 μl vs. 20 μl) to attain a similar magnitude of secondary thermal hyperalgesia. 20 μl dose of mustard oil produced severe painful responses in ProE females, including abdominal writhing and escape attempts, resulting in a lack of tail flick response. These observations are consistent with a previous report of the use of three fold higher dose of capsaicin in males vs. females to produce a comparable magnitude of hyperalgesia [1]. Hence, mustard oil-induced secondary hyperalgesia appears to have a sex-specific effect that may be attributable to sex-related differences in the descending facilitation of pain mechanisms. It is noteworthy that previous studies using OFQ have failed to detect any sex-related differences in OFQ antinociception which is contradictory to the findings presented here. In a study [28], Mogil and colleagues observed no significant sex-related differences in pain modulation after supraspinally injected OFQ and therefore reported data combined from both sexes. However, in this study conducted in mice (vs. rats in the present study), females were not separated based on their estrous cycle stage. Estrogen levels peak during proestrous and are low at diestrous stage. We have shown here as well as before [5, 10,32,38] that it is the presence of high levels of estrogen in females (at proestrous stage or in estrogen replaced OVX rats) that attenuates analgesia induced by ORL_1 or α_2-adrenoceptors; diestrous or OVX females showed robust analgesia similar to that in males. Hence, one might overlook sex-related differences in data pooled from randomly cycling females. Further, drug responses are known to vary depending on the genotype [27].

Supraspinal, but not i.t. administration of OFQ has been shown to have pronociceptive effects. We previously reported a dose dependent antinociception by OFQ in male and OVX rats but regardless of the dose (2.5 – 10 nmol; i.t.), OFQ remained ineffective in OVX+E group [5]. Hence, the available data doesn't support the view that estrogen may "flip a neural switch" from antinociceptive to pronociceptive action of spinal OFQ.

Our finding that estrogen alters the antihyperalgesic effect of OFQ in the female indicates the dependence of the therapeutic potential of this opioid peptide on the hormonal status i.e. estrous cycle stage, premenopausal versus postmenopausal. Estrogen-induced reduction in the ORL_1 receptor mediated inhibition of nociception may make women, during reproductive years, more susceptible to the development of pain syndromes. Further, our finding that OFQ is effective against mustard oil-induced secondary thermal hyperalgesia, follows the general consensus that OFQ, administered intrathecally, produces antinociception in males [5,15,16,23,24,29, 36] and extends the possibility for use of this fairly novel opioid as an effective antinociceptive agent for instances of chronic pain in men and in post-menopausal women.

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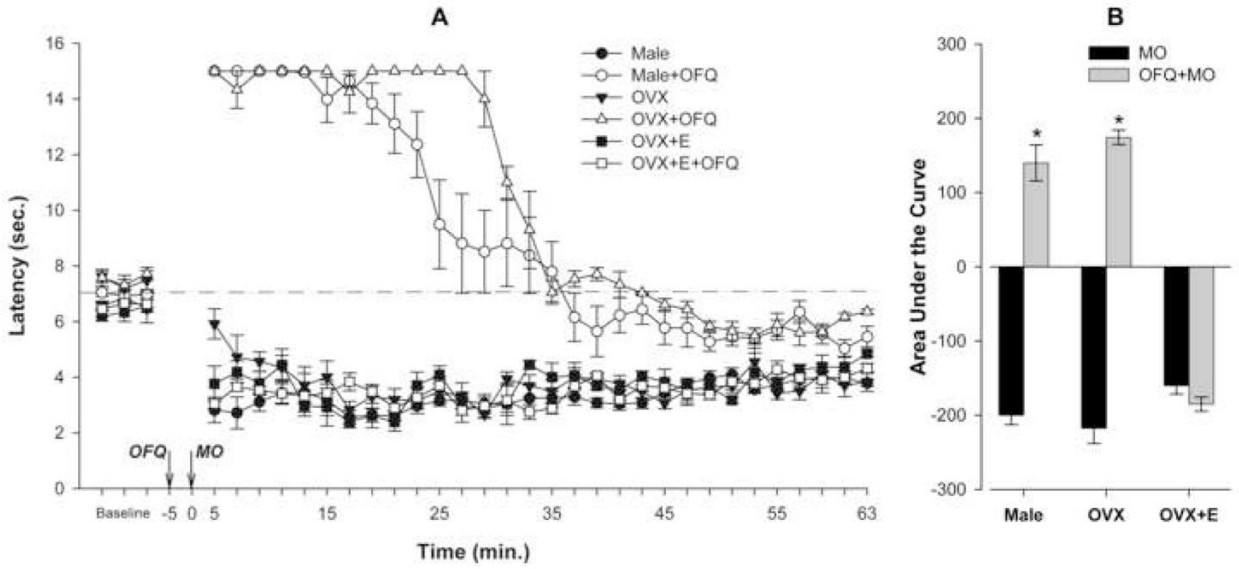

Figure 1.

A. OFQ reverses mustard oil (MO)-induced secondary thermal hyperalgesia and produces antinociception in male, and OVX animals but not in OVX+E animals. A repeated measures ANOVA conducted on tail flick latencies yielded significant main effects of Group ($F_{(2,18}$= 59.56; $p < 0.001$), Time ($F_{(32–576)}$= 29.73; $p < 0.001$), Drug ($F_{(1,18)}$= 239.19; $p < 0.001$), and interactions between Group × Drug ($F_{(2,18)}$= 60.67; $p < 0.001$) and Group × Time ×Drug ($F_{(64,576)}$= 11.18; $p < 0.001$). *Post hoc* comparisons indicated that OFQ (given 5 min. prior to mustard oil application) produced significant increases in tail-flick latencies in males (n=6) and OVX females (n=3; beginning at time 5 min) as compared to baseline latencies and mustard oil control latencies with respect to each group (all $p < 0.05$). Also, mustard oil alone (control) produces robust secondary thermal hyperalgesia in male, OVX, and OVX+E animals (n=4/gp) as evident by the significant decrease in tail-flick latencies from baseline of between 6 and 8 seconds (all $p < 0.05$). The antihyperalgesic/antinociceptive effects of OFQ persisted for up to 30 min. However, OFQ failed to modulate mustard oil-induced secondary thermal hyperalgesia in OVX+E females (n=4). **B.** OFQ is a substantially effective antihyperalgesic/antinociceptive agent, inhibiting mustard oil induced secondary thermal hyperalgesia and producing antinociception in male and OVX rats but not in OVX+E rats. ANOVA of the area under the curve yielded significant main effect of Group ($F_{(5,23)}$ = 89.73; $p < 0.001$). *Post hoc* comparisons revealed that OFQ produced significant antihyperalgesic effects in males and OVX females against mustard oil-induced secondary thermal hyperalgesia as compared to control groups (all $p < 0.05$). In contrast, the antihyperalgesic effects of OFQ were not observed in the estradiol-treated OVX+E group. *p<0.05.

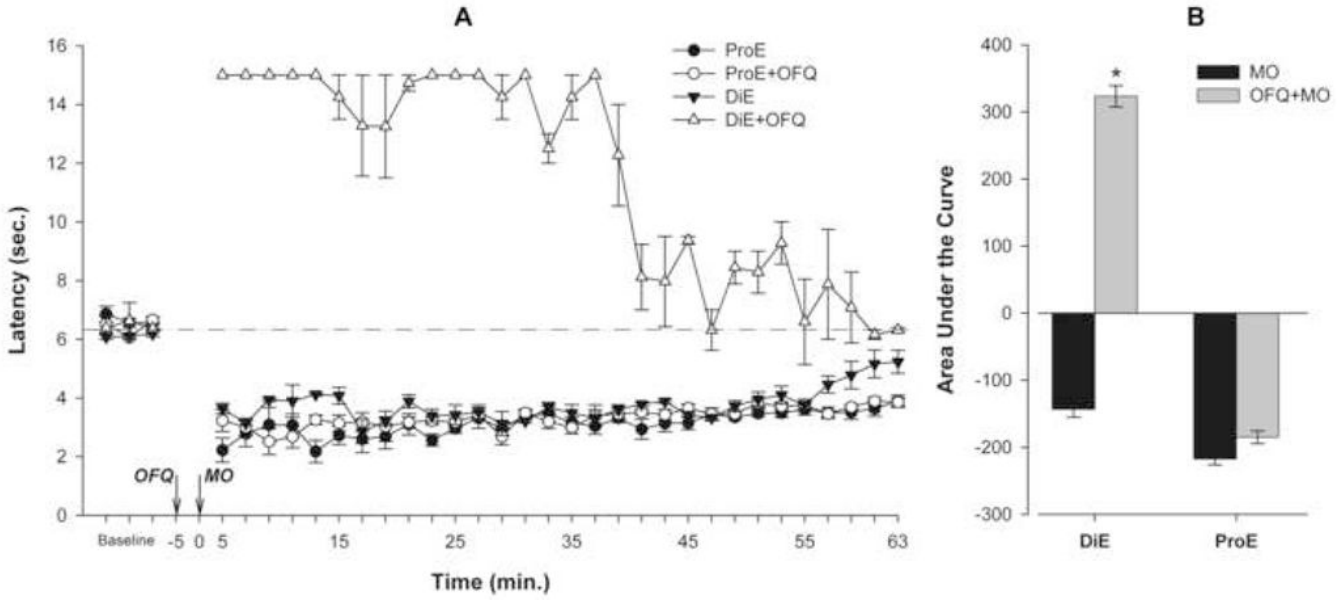

Figure 2.

A. OFQ reverses mustard oil-induced secondary thermal hyperalgesia and produces antinociception in normally cycling females at the diestrous (low levels of circulating estrogen) but not the proestrous (high levels of circulating estrogen) stage of the estrous cycle. A repeated measures ANOVA conducted on tail flick latencies yielded significant main effects of Group ($F_{(1,11)}$= 2188; $p < 0.001$), Time ($F_{(32–352)}$= 13.56; $p < 0.001$), Drug ($F_{(1,11)}$= 1807; $p < 0.001$), and interactions between Group × Drug ($F_{(1,11)}$= 1650; $p < 0.001$) and Group × Time × Drug ($F_{(32,352)}$= 31.85; $p < 0.001$). *Post hoc* analysis comparisons revealed that OFQ (given 5 min prior to MO) significantly increased tail-flick latencies starting at time 5 minutes in DiE females ($p < 0.05$), but not in ProE females (n=4/gp). *Post hoc* analysis also indicated that mustard oil alone significantly facilitated tail-flick latencies in proestrous (n=4) and diestrous (n=3) groups (all $p < 0.05$). **B.** OFQ proves to be substantially effective antihyperalgesic/antinociceptive agent in DiE, but not in ProE rats. ANOVA of the area under the curve yielded significant main effect of Group ($F_{(3,14)}$ = 481; $p < 0.001$). *Post hoc* comparisons revealed that OFQ produced significant antihyperalgesic and antinociceptive effects in DiE females against mustard oil-induced secondary thermal hyperalgesia as compared to control and ProE groups (all $p < 0.05$). In contrast, the antihyperalgesic effects of OFQ were not observed in ProE females. * $p < 0.05$.