Published in final edited form as:
Neuroscience. 2011 September 8; 190: 379–385. doi:10.1016/j.neuroscience.2011.05.062.

Activation of peripheral delta–opioid receptors leads to anti-hyperalgesic responses in the masseter muscle of male and female rats

Jami L. Saloman, **Katelyn Y. Niu**, and **Jin Y. Ro**[+]
University of Maryland Baltimore School of Dentistry, Department of Neural and Pain Sciences, Program in Neuroscience, 650 W. Baltimore Street, Baltimore, Maryland 21201, USA

Abstract

In this project, we examined peripheral δ-opioid receptor (DOR)-mediated anti-hyperalgesic responses in the context of an acute orofacial muscle pain condition in both male and female rats. We also investigated whether the ATP-sensitive K^+ channel (KATP), a downstream target of OR signaling, contributes to DOR-mediated anti-hyperalgesic responses. Local pretreatment of the masseter with a DOR agonist, DPDPE, dose-dependently attenuated capsaicin-induced mechanical hypersensitivity in both male and female rats. However, there were sex differences in the potency of local DPDPE in that a 10 fold higher dose of DPDPE was required in female rats to produce the level of anti-hyperalgesia achieved in male rats. The sex differences in the DPDPE effect may not be fully explained by DOR expression level since there was no significant sex difference in DOR mRNA levels in trigeminal ganglia (TG). Finally, pretreatment of the masseter with the KATP antagonist, glibenclamide significantly blocked the effects of DPDPE in male rats suggesting that the peripheral DOR effect is mediated by the KATP. These studies revealed novel information about sex differences with regards to peripherally localized DOR-mediated anti-hyperalgesia under an orofacial muscle pain condition.

Keywords

trigeminal; muscle pain; sex differences; potassium channels; Sprague Dawley rats

1. Introduction

The functional role of peripheral opioid receptors (ORs) in attenuating pain and hyperalgesia has been demonstrated for decades (Ferreira et al., 1979a,b; Sachs et al., 2004; Stein et al., 2003), and an overwhelming amount of animal data supporting the role of peripheral ORs under various pain conditions is continuously being accumulated (Garlicki et al., 2006; Guan et al., 2008; Núñez et al., 2007; Obara et al., 2009). Consistent with the animal data, pain relief from local application of opioids has been reported in patients with chronic

[+]**Send correspondence and proofs to:** Dr. Jin Y. Ro, Department of Neural and Pain Sci, Program in Neuroscience, University of Maryland Baltimore, School of Dentistry, 650 W. Baltimore St., Baltimore, Maryland 21201, USA, Tel.: (410) 706-6027, Fax: (410) 706-4172, jro@umaryland.edu.

There are no conflicts of interest associated with the present study.

rheumatoid and osteoarthritis, ischemic pain, dental pain, pancreatitis, and postoperative visceral pain (Dionne et al., 2001; Duckett et al., 1997; Eisenach et al., 2003; Keskinbora et al., 2009; Likar et al., 2001; Modi et al., 2009; Rorarius et al., 1999).

While all three major subtypes of opioid receptors, namely, μ–, δ–, and κ–opioid receptors (MORs, DORs, KORs, respectively) have been implicated in peripheral analgesia and/or anti-hyperalgesia each subtype of OR may be associated with distinct regulatory mechanisms. Therefore, they may provide distinct therapeutic advantages in different pain conditions. There is evidence that direct activation of peripheral DORs leads to potent anti-hyperalgesic effects under inflammatory and neuropathic pain conditions (Kabli et al., 2007; Pacheco et al., 2005; Shinoda et al., 2007; Stein et al., 1989). However, in comparison to MORs and KORs, the role of peripheral DORs is relatively under studied, and the role of peripheral DORs in a muscle pain condition has never been demonstrated.

While sex differences in spinally- and supraspinally-mediated opioid analgesia have been documented few studies have examined sex differences in peripheral OR-mediated analgesia (Bodnar et al., 2010; Craft 2003; Flores et al., 2003). In a visceral pain model, activation of peripheral MORs produces more potent analgesia in male rats than in females (Ji et al., 2006). Similarly, local morphine in the temporomandibular joint (TMJ) of male rats, but not females, significantly reduces glutamate-evoked jaw muscle activity (Cai et al., 2001). However, a specific KOR agonist administered in the TMJ produces a greater reduction of formalin-induced nociceptive responses in female rats (Clemente et al., 2004). Sex differences in peripheral DOR-mediated analgesia have not been described.

Specific agonists for ORs open inwardly rectifying K^+ channels through the activation of $G_{i/o}$ proteins in neurons (North et al., 1987); one of which is the ATP-sensitive K^+ channel (KATP). Activation or blockade of the KATP in sensory neurons modulates the anti-hyperalgesic responses induced by all three subtypes of ORs in the spinal system (Amarante et al., 2004; Pacheco and Duarte 2005). While both pore-forming and regulatory subunits of KATP are expressed in trigeminal sensory neurons (Niu et al., 2011) the functional interaction between KATP and ORs in the orofacial model has not been demonstrated.

These observations have led us to investigate (1) whether activation of peripheral DORs effectively attenuates capsaicin-induced mechanical hypersensitivity in the masseter muscle, (2) whether there are sex differences in peripheral DOR responses, and (3) whether the anti-hyperalgesic responses of peripheral DORs involves the KATP.

2. Experimental Procedures

2.1 Animals

Age matched adult male and female Sprague-Dawley rats (8 weeks old; 250–300g for males and 225–260g for females; Harlan, Indianapolis) were used in this experiment. All animals were housed in a temperature-controlled room under a 12:12 light-dark cycle with access to food and water ad libitum. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and under a University of Maryland approved Institutional Animal Care and Use Committee protocol. Estrus cycle in females rats was not determined in this study.

2.2 Real-Time RT-PCR

To quantitatively compare DOR mRNA between male and female TG, Real-Time RT-PCR was performed. Total RNA was extracted from TG with Trizol (Sigma) and purified according to the RNeasy kit (Qiagen) that included a DNase treatment to remove genomic DNA. Reverse transcription was carried out using the Superscript First strand synthesis kit

(Invitrogen). SuperScript II (Invitrogen) was used to generate cDNA from 1μg of RNA along with 2.5ng of random primer per reaction. Real-time PCR analysis of cDNA equal to 25ng of RNA was then performed using Maxima SYBR Green/ROX qPCR Master Mix (Fermentas) in an Eppendorf Mastercycler ep realplex 2.0. The following primers for DOR were used: sense 5'-TGGGTCTTGGCTTCAGGTGT-3', antisense 5'-CGTGCATACCACTGCTCCAT-5'.

2.3 Drug preparation and administration

Capsaicin (Sigma) was dissolved in ethanol (23%), Tween 80 (7%) and phosphate buffer solution (PBS) (70%). DPDPE (Tocris Cookson) was dissolved in PBS. PBS was 0.01M phosphate, 0.14M NaCl, and 3mM KCl, pH7.4. Glibenclamide (Tocris Cookson) was dissolved in DMSO. All drugs were administered intramuscularly into the masseter muscle. In order to make sure that the drugs and their corresponding vehicles were administered in the same target region of the muscle the injection site was determined by palpating the masseter muscle between the zygomatic bone and the angle of the mandible. Injections were made with a 27-gauge needle. Upon contacting the mandible the needle was slowly withdrawn into the mid-region of the masseter and injections were made for 5–10 seconds.

2.4 Behavioral studies

It is well established that noxious chemical or mechanical stimulation of the masseter muscle evokes characteristic shaking of the ipsilateral hindpaw in lightly anesthetized rats (Han et al., 2008; Ro et al., 2003; Sánchez et al., 2010). We have previously described the use of this behavior for testing mechanical sensitivity of the masseter muscle in rats (Ro et al., 2007, 2009).

Since pentobarbital metabolism is different between male and female rats, we measured the heart rate of both under different anesthetic regimens in order to identify conditions that elicit comparable physiological responses. It was determined that male rats would receive an initial intraperitoneal injection of 40mg/kg and female rats 35mg/kg of sodium pentobarbital for the behavioral studies. A tail vein was connected to an infusion pump (Harvard Apparatus, Pump11) for continuous infusion of pentobarbital.

A level of 'light' anesthesia was determined by providing a noxious pinch to the tail or the hindpaw with a serrated forceps. Male rats typically respond to the noxious pinch on the tail with an abdominal contraction and with a withdrawal reflex to the noxious pinch of a hindpaw about 15min after the initial anesthesia. It typically took about 30–45min for female rats to show similar responses. Once the animal reached this level a metal clip calibrated to produce 600g of force was applied 5 consecutive times. Experiments were initiated only after the animals showed reliable reflex responses to every clip application regardless of the sex of the animal. During the course of behavioral experiment male rats required additional anesthetic, which was provided via the tail vein. The rate of infusion was adjusted to maintain a relatively light level of anesthesia throughout the duration of the experiment (3mg/hr). Female rats did not require additional pentobarbital.

During the behavioral observation a baseline mechanical threshold for evoking the hindpaw responses was determined 15min prior to drug injection using the electronic von Frey (VF) anesthesiometer (IITC Life Science, Inc, Woodland Hills, CA). A rigid tip (diameter 2mm) attached to the VF meter was applied to the masseter muscle until the animals responded with hindpaw shaking. The animal's head was rested flat against the surface of the table when pressing the anesthesiometer on the masseter in order to provide stability. The threshold was defined as the lowest force necessary to evoke the hindpaw response. Changes in masseter sensitivity were then assessed at 15, 30, 45, 60 and 90min following drug

treatments. We calculated percent changes in VF thresholds following drug treatment with respect to the baseline threshold and plotted against time. In order to assess the overall magnitude of drug-induced changes in masseter sensitivity over time, the area under the curve (AUC) was calculated for the normalized data for each rat using the trapezoid rule. All behavioral observations were made by one experimenter who was blinded to the experimental conditions in order to maintain the consistency of assessing behavioral responses. All animals were kept warm throughout the experiments with thermal blankets.

2.5 Experimental and control groups for behavioral studies

To examine whether activation of peripheral DORs blocks capsaicin-induced mechanical hypersensitivity, the masseter muscle was pretreated with a specific agonist for the DOR, [D-Pen^2,D-Pen^5]-Enkephalin (DPDPE) (1, 10, 100, and 300μg/50μl) or the vehicle, PBS, 10min prior to capsaicin (0.1%, 100μl) injection in both male and female rats. Another group of rats was treated with a selective DOR antagonist, naltrindole (100μg/20μl) prior to the injection of DPDPE (100μg) in order to test the receptor specific action of DPDPE. The doses of DPDPE were adapted from a published study (Stein et al., 1989). Since it is possible that high doses of naltrindole can block other opioid receptors we chose a dose of 100μg (≈0.33mg/kg) which is 20 times lower than the dose (20mg/kg, s.c.) shown to successfully antagonize the effects of the selective DOR agonist [D-Ser^2, Leu^5, Thr^6]Enkephalin without blocking the antinociceptive effects of the KOR or MOR agonists morphine and U50488H, respectively (Portoghese et al., 1988).

There is a possibility that DPDPE injected into the masseter can mediate its effects by activating central DORs. To evaluate possible systemic effects, the highest dose of DPDPE (300μg) was administered into the masseter muscle contralateral to the capsaicin injection in a separate group of animals. In order to investigate whether the peripheral DOR-mediated anti-hyperalgesia involves the KATP a specific KATP antagonist, glibenclamide (100μg/20μl), was administered prior to DPDPE and capsaicin treatments in the masseter muscle of male rats. The highest dose of each drug used in this study was administered in the masseter contralateral to the capsaicin treatment to rule out the possibility of systemic effects. Rats were randomly assigned to experimental and control groups, and each group consisted of 6–10 rats.

2.6 Data Analysis

For Real-Time RT-PCR analysis, the amount of DOR mRNA was normalized to the amount of GAPDH mRNA. Relative quantification of the mRNA was calculated by the comparative Ct method (ΔΔCt method), and a t-test was used to compare males and females. The nature of this method is to automatically normalize data to a chosen control group. In our case, we chose to normalize the female data to male, which were set to 100%. The ΔΔCt method calculates changes in gene expression as a relative fold difference between the experimental and the control samples. The cycle threshold, Ct, was determined as the cycle at which the fluorescence from a sample crossed the threshold level. Delta Ct was the difference between the Ct values of the experimental gene (DOR) and its internal control gene (GAPDH). ΔΔCt was calculated as the difference of the two delta Ct values. The relative amount of DOR mRNA in the experimental condition compared to the control condition was calculated as $2^{-\Delta\Delta Ct}$.

For behavioral studies, the time-dependent mean percent changes in mechanical thresholds were normalized to the baseline threshold and analyzed with a two-way ANOVA with repeated measures. In addition, either the student t-test or one-way ANOVA was used to evaluate the overall magnitude of mechanical hypersensitivity assessed as the area under the curve (AUC), which was calculated from the normalized data for each rat. All multiple

group comparisons were followed by a post hoc test (Dunnett's or Bonferroni's). The significance of all statistical analyses was set at $p<0.05$, and data are presented as mean ±SE.

3. Results

In this study we utilized the lightly anesthetized rodent behavioral model that was specifically designed for testing craniofacial muscular sensitivity. Since anesthetic effects of pentobarbital are different between male and female rats (Craft and Leitl, 2006) we performed two sets of preliminary experiments to ensure that the animals were maintained under similar anesthetic planes during the behavioral testing. First, we compared the changes in heart rate between male and female rats over the time course of the behavioral testing following the initial anesthesia. A single dose of pentobarbital (40mg/kg) administered in male rats kept the animals anesthetized for approximately 60min, during which the heart rates were maintained above 300beats/min (Data not shown). The heart rate recording was stopped after 60min as the animals quickly came out of the anesthesia. Since our behavioral paradigm is at least 90min additional anesthesia was required. When the same initial dose of anesthesia was accompanied by continuous infusion of additional pentobarbital (3mg/hr) via the tail vein the heart rate was reduced by an average 116 beats/min over the 90min of testing.

In contrast to male rats, the initial anesthesia, a lower dose of pentobarbital (35mg/kg), kept the female rats anesthetized with changes in heart rate comparable to those observed in male rats that received the continuous infusion of the anesthetic. We observed a gradual decline in heart, the peak reduction being an average 126 beats/min over the 90min of testing. There was no significant difference in heart rate across the recording period between male and female rats. Thus, different anesthetic regimens in male (40mg/kg i.p. plus infusion) and female (35mg/kg i.p.) rats produced similar changes in heart rates. The corneal and withdrawal reflexes were intact in all animals throughout the experiment.

Second, we measured baseline mechanical thresholds of the masseter muscle in separate groups of male and female rats under the same anesthetic regimens described above. The baseline mechanical thresholds ranged between 500–600g as we have previously published (Lee and Ro, 2007). There was no significant difference between the baseline mechanical threshold of male and female rats ($t=2.018$, $p=0.060$; Fig 1A). Therefore, along with our routine monitoring of reflex responses during the experiment, these data provide additional support that we were able to maintain male and female rats under comparable anesthetic levels during the behavioral experiments.

As we have shown previously (Ro et al., 2009), an intramuscular injection of capsaicin produced a reduction of the mechanical thresholds as early as 15min, which then gradually returned to the baseline level in 90min (Fig 1B). There was no significant difference in the capsaicin-induced reduction in mechanical thresholds over time between male and female rats ($F=0.129$, $p=0.724$; Fig 1B). To examine the overall magnitude of the capsaicin effect, irrespective of time we calculated the area under the curve (AUC) for Fig 1B and found there was no significant difference between the AUC of capsaicin induced hypersensitivity between male and female rats ($t=0.497$, $p=0.626$; Fig 1C).

The masseter muscle was pretreated with the DOR agonist DPDPE to assess the role of peripheral DORs in anti-hyperalgesia. In male rats the capsaicin-induced mechanical hypersensitivity was significantly and dose-dependently attenuated by DPDPE ($F=9.241$, $p<0.001$; Fig 2A). The AUC was almost completely prevented with 10μg of DPDPE (Fig 2B). At higher doses (100 and 300μg) DPDPE produced slight analgesic responses. Pretreatment with a selective DOR antagonist, naltrindole (100μg) prevented the anti-

hyperalgesic effect of DPDPE, indicating that the effect is mediated specifically by DORs (t=−8.923, $p<0.001$; Fig 2C).

The same doses of DPDPE used in males also produced significant dose-dependent responses in female rats (F=51.671, $p<0.001$; Fig 2D). However, DPDPE at the lowest dose (1μg) actually produced significantly more mechanical hypersensitivity compared to vehicle. This indicates that females are not necessarily exhibiting more capsaicin induced hypersensitivity, but that DPDPE at low doses may be driving sensitivity in a pro-nociceptive manner. A dose of DPDPE which completely blocked the capsaicin-induced mechanical hypersensitivity in male rats (10μg) was ineffective in females (Fig 2E). Only at higher doses did DPDPE significantly attenuate the mechanical hypersensitivity. When the highest dose of DPDPE (300μg) was administered in the masseter contralateral to the capsaicin injection site it failed to block the mechanical hypersensitivity, suggesting that even 300μg of DPDPE does not produce systemic effects (t=10.294, $p<0.001$) (data not shown). Thus, peripheral application of DPDPE exhibited significant sex differences in attenuating the overall magnitude of capsaicin-induced mechanical hypersensitivity in the masseter muscle (F=15.03, $p<0.001$; Fig 2F). The dose-effect curve illustrates a leftward shift in the responses in males compared to females. The IC50 values calculated based on the AUC data were 2.7μg and 20μg for male and female rats, respectively. These data reveal that peripheral DPDPE is about 10 times more potent in male rats compared to females.

It is well known that opioid receptor antibodies are not always reliable. Although recent studies demonstrate DOR immunoreactivity in sensory neurons (Wang et al., 2010) the issues related to antibody specificity remain controversial (Scherrer et al., 2009). The specificity of DOR antibodies in western blot experiments has not been clearly demonstrated. In our hands, several commercially available antibodies for western blot failed to be validated in DOR KO tissue. Therefore, to establish whether differential DOR expression underlies the observed sex difference, we measured DOR mRNA. There was no significant difference in the DOR mRNA expression from whole TG between naïve male and female rats (t=−1.760, $p=0.117$; Fig 3).

Finally, we investigated whether peripheral DOR-mediated attenuation of mechanical hypersensitivity involves KATP. We injected a specific antagonist for the KATP, glibenclamide (100μg) prior to DPDPE (100μg) into the masseter muscle of male rats. The anti-hyperalgesic effect of DPDPE was blocked when glibenclamide was pre-administered into the same muscle (F=24.719, $p<0.001$; Fig 4). The same dose of glibenclamide given in the contralateral masseter did not block the DPDPE effect indicating that glibenclamide produced its effect via antagonizing local KATPs. The injection of glibenclamide by itself did not alter the mechanical sensitivity of the masseter muscle (data not shown).

4. Discussion

Accumulating studies provide a compelling rationale for targeting peripheral ORs as a novel treatment for various types of pain (Keskinbora et al., 2009). Peripheral application of DOR agonists produces potent anti-hyperalgesic effects under inflammatory and neuropathic pain conditions (Kabli et al., 2007; Pacheco et al., 2005; Shinoda et al., 2007; Stein et al., 1989). As with peripheral MORs the efficacy of a DOR agonist is not readily detectable in normal tissue, but it is greatly augmented under conditions of tissue injury and inflammation (Kabli et al., 2007; Stein et al., 1989). The increase in peripheral DOR efficacy can be explained, in part, by the increased expression of DORs in dorsal root ganglia (DRG) as well as trafficking of the receptor protein to the site of injury under a nerve injury condition (Kabli et al., 2007). However, unlike MORs, peripheral DORs are not significantly up-regulated under inflammatory conditions (Ji et al., 1995; Obara et al., 2009). Also, since the time

course of increased OR synthesis and transport does not always correlate with increased opioid effects, which could appear within minutes, other mechanisms such as stimulus-induced insertion of ORs to the plasma membrane could play a greater role in increased peripheral DOR efficacy (Gendron et al., 2006).

Inflammatory substances such as bradykinin, arachidonic acid and proteases rapidly increase the functional competence of DORs in sensory neurons by trafficking the receptor proteins to the plasma membrane (Patwardhan et al., 2006; Rowan et al., 2009). A local injection of capsaicin has also been shown to rapidly increase the cell surface availability of DORs in DRG neurons (Gendron et al., 2006). Our data showing the reduction of mechanical hypersensitivity as early as 15min upon DPDPE administration suggest that acute myositis induced by capsaicin might be promoting functional competency of DORs, thus increasing the efficacy of DPDPE for attenuating hyperalgesic responses.

Of the studies that show anti-hyperalgesic effects of peripheral DORs in rodents, to the best of our knowledge, there is no data that directly assess sex differences (Hervera et al., 2009; Kabli et a., 2007; Leanez et al., 2009; Obara et al., 2009; Pacheco and Duarte 2005; Pacheco et al., 2005; Pena-dos-Santos et al., 2009; Stein et al., 1989). Our data showed that a 10 fold higher dose of DPDPE was required to produce anti-hyperalgesic responses in female rats. Thus, peripheral DOR responses observed in one sex may not be easily generalized to both sexes. Our data also imply that the endogenous opioid peptides released under inflammatory conditions may produce different responses between the two sexes. A low level of endogenous opioid peptides that activate peripheral DORs can produce anti-hyperalgesic responses in males, but may produce opposite effects in females.

The sex difference is likely to be modulated by sex hormones, but the data on their influence on peripheral ORs is limited. KORs in the TMJ produce greater anti-nociceptive responses during diestrus compared to proestrus phase (Clemente et al., 2004). Those authors suggested that a high level of estrogen attenuates KOR-mediated effects. In our data, the SE were not appreciably higher in females relative to males suggesting that cycling estrogen has little effect on the DOR responses. However, in order to obtain more precise information about the role of sex hormones in DOR responses, additional studies with female rats of known estrus stage or gonadectomized rats need to be performed.

The expression of sex differences in DOR-mediated responses may involve multiple mechanisms. In the CNS, sexual dimorphism in the density of ORs provides an anatomical basis for sex differences in opioid-mediated behaviors (Carretero et al., 2004; Flores et al., 2003; Harris et al., 2004). Thus, it is possible that sex differences in the expression level of DORs in sensory neurons could serve as an underlying basis for sex differences in DPDPE effects. In our study, however, there was no significant difference between the levels of DOR mRNA in male and female rats suggesting the sex differences in DOR-mediated responses result from mechanisms other than DOR expression. It is possible that we observed no differences because mRNA was measured from entire TG as opposed to specifically masseter afferents. However, another possibility is the sex difference results from differences in the downstream targets of DORs. ORs have been linked to adenylate cyclase, potassium channels (e.g. GIRKs and KATPs), and voltage gated calcium channels all of which could contribute to the observed DOR-mediated sex difference (Standifer et al., 1997). We recently reported that there is significantly greater expression of the KATP subunit Kir6.2 in male TG compared to females (Niu et al., 2011).

We showed that the DOR-mediated anti-hyperalgesia was prevented in the presence of glibenclamide, a KATP antagonist, indicating that the KATP is required for DOR function in our model. Therefore, we further corroborate recent findings in various pain models that

local activation or blockade of KATPs modulates the anti-hyperalgesic effects induced by peripheral opioid receptors (Amarante et al., 2004; Granados-Soto et al., 2002; Pacheco and Duarte 2005; Picolo et al., 2003; Rodrigues et al., 2000).

Taken together, our data suggest that there is merit in pursuing the development of DOR agonists, especially since targeting this subtype of OR has distinct advantages over others such as reduced physical dependence, gastrointestinal dysfunction as well as respiratory depression (Cheng et al., 1993; Cowan et al., 1988; Sheldon et al., 1990). Since DPDPE is primarily a delta-1 receptor agonist the possibility of the involvement of delta-2 receptors still needs to be pursued (Porreca et al., 1992). The results from this study should offer important new insights for the development of mechanism-based sex specific pharmacological treatment alternatives that can be directed at the peripheral OR system to ameliorate muscle pain conditions, such as temporomandibular disorders.

Research Highlights

- Peripheral δ opioid receptors (DORs) mediate anti-hyperalgesic responses under acute myositis.
- Peripheral DORs produce more potent responses in male than female rats.
- There are no sex differences in the basal level of DOR expression in trigeminal ganglia (TG).
- The effect of peripheral DORs is mediated via ATP-dependent K^+ channels.

Abbreviations

AUC	Area under curve
DOR	Delta (δ) opioid receptor
DRG	Dorsal root ganglia
KATP	ATP sensitive potassium channels
KOR	kappa (κ) opioid receptor
MOR	mu (μ) opioid receptor
TG	Trigeminal ganglia
VF	Von Frey

Acknowledgments

We thank Mr. Gregory Haynes for his technical assistance in behavioral experiments. This study was supported by NIH grant RO1 DE19448 (JYR).

References

Amarante LH, Alves DP, Duarte ID. Study of the involvement of K+ channels in the peripheral antinociception of the kappa-opioid receptor agonist bremazocine. Eur J Pharmacol. 2004; 494:155–160. [PubMed: 15212969]

Bodnar RJ, Kest B. Sex differences in opioid analgesia, hyperalgesia, tolerance and withdrawal: Central mechanisms of action and roles of gonadal hormones. Horm Behav. 2010; 58:72–81. [PubMed: 19786031]

Cai BB, Cairns BE, Sessle BJ, Hu JW. Sex-related suppression of reflex jaw muscle activity by peripheral morphine but not GABA. Neuroreport. 2001; 12:3457–3460. [PubMed: 11733690]

Carretero J, Bodego P, Rodriguez RE, Rubio M, Blanco E, Burks DJ. Expression of the mu-opioid receptor in the anterior pituitary gland is influenced by age and sex. Neuropeptides. 2004; 38:63–68. [PubMed: 15223267]

Cheng PY, Wu D, Decena J, Soong Y, McCabe S, Szeto HH. Opioid-induced stimulation of fetal respiratory activity by [D-Ala2]deltorphin I. Eur J Pharmacol. 1993; 230:85–88. [PubMed: 8381356]

Clemente JT, Parada CA, Veiga MC, Gear RW, Tambeli CH. Sexual dimorphism in the antinociception mediated by kappa opioid receptors in the rat temporomandibular joint. Neurosci Lett. 2004; 372:250–255. [PubMed: 15542250]

Cowan A, Zhu XZ, Mosberg HI, Omnaas JR, Porreca F. Direct dependence studies in rats with agents selective for different types of opioid receptor. J Pharmacol Exp Ther. 1988; 246:950–955. [PubMed: 2901490]

Craft RM. Sex differences in opioid analgesia: "from mouse to man". Clin J Pain. 2003; 19:175–186. [PubMed: 12792556]

Craft RM, Leitl MD. Potentiation of morphine antinociception by pentoparbital in female vs. male rats. Pain. 2006; 121(1–2):115–125. [PubMed: 16473463]

Dionne RA, Lepinski AM, Gordon SM, Jaber L, Brahim JS, Hargreaves KM. Analgesic effects of peripherally administered opioids in clinical models of acute and chronic inflammation. Clin Pharmacol Ther. 2001; 70:66–73. [PubMed: 11452246]

Duckett JW, Cangiano T, Cubina M, Howe C, Cohen D. Intravesical morphine analgesia after bladder surgery. J Urol. 1997; 157:1407–1409. [PubMed: 9120965]

Eisenach JC, Carpenter R, Curry R. Analgesia from a peripherally active kappa-opioid receptor agonist in patients with chronic pancreatitis. Pain. 2003; 101:89–95. [PubMed: 12507703]

Ferreira SH, Nakamura M. II - prostaglandin hyperalgesia: The peripheral analgesic activity of morphine, enkephalins and opioid antagonists. Prostaglandins. 1979a; 18:191–200. [PubMed: 230543]

Ferreira SH, Nakamura M. III - prostaglandin hyperalgesia: Relevance of the peripheral effect for the analgesic action of opioid-antagonists. Prostaglandins. 1979b; 18:201–208. [PubMed: 523678]

Flores CA, Shughrue P, Petersen SL, Mokha SS. Sex-related differences in the distribution of opioid receptor-like 1 receptor mRNA and colocalization with estrogen receptor mRNA in neurons of the spinal trigeminal nucleus caudalis in the rat. Neuroscience. 2003; 118:769–778. [PubMed: 12710984]

Garlicki J, Dorazil-Dudzik M, Wordliczek J, Przewlocka B. Effect of intraarticular tramadol administration in the rat model of knee joint inflammation. Pharmacol Rep. 2006; 58:672–679. [PubMed: 17085859]

Gendron L, Lucido AL, Mennicken F, O'Donnell D, Vincent JP, Stroh T, Beaudet A. Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia. J Neurosci. 2006; 26:953–962. [PubMed: 16421315]

Granados-Soto V, Arguelles CF, Ortiz MI. The peripheral antinociceptive effect of resveratrol is associated with activation of potassium channels. Neuropharmacology. 2002; 43:917–923. [PubMed: 12384177]

Guan Y, Johanek LM, Hartke TV, Shim B, Tao YX, Ringkamp M, Meyer RA, Raja SN. Peripherally acting mu-opioid receptor agonist attenuates neuropathic pain in rats after L5 spinal nerve injury. Pain. 2008; 138:318–329. [PubMed: 18276075]

Han SR, Lee MK, Lim KH, Yang GY, Jeon HJ, Ju JS, Yoon YW, Kim SK, Ahn DK. Intramuscular administration of morphine reduces mustard-oil-induced craniofacial-muscle pain behavior in lightly anesthetized rats. Eur J Pain. 2007; 12(3):361–370. [PubMed: 17768078]

Harris JA, Chang PC, Drake CT. Kappa opioid receptors in rat spinal cord: Sex-linked distribution differences. Neuroscience. 2004; 124:879–890. [PubMed: 15026128]

Hervera A, Leanez S, Negrete R, Pol O. The peripheral administration of a nitric oxide donor potentiates the local antinociceptive effects of a DOR agonist during chronic inflammatory pain in mice. Naunyn Schmiedebergs Arch Pharmacol. 2009; 380:345–352. [PubMed: 19636536]

Ji RR, Zhang Q, Law PY, Low HH, Elde R, Hokfelt T. Expression of mu-, delta-, and kappa-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. J Neurosci. 1995; 15:8156–8166. [PubMed: 8613750]

Ji Y, Murphy AZ, Traub RJ. Sex differences in morphine-induced analgesia of visceral pain are supraspinally and peripherally mediated. Am J Physiol Regul Integr Comp Physiol. 2006; 291:R307–R314. [PubMed: 16556902]

Kabli N, Cahill CM. Anti-allodynic effects of peripheral delta opioid receptors in neuropathic pain. Pain. 2007; 127:84–93. [PubMed: 16963185]

Keskinbora K, Aydinli I. Perineural morphine in patients with chronic ischemic lower extremity pain: Efficacy and long-term results. J Anesth. 2009; 23:11–18. [PubMed: 19234816]

Leanez S, Hervera A, Pol O. Peripheral antinociceptive effects of mu- and delta-opioid receptor agonists in NOS2 and NOS1 knockout mice during chronic inflammatory pain. Eur J Pharmacol. 2009; 602:41–49. [PubMed: 19041302]

Lee JS, Ro JY. Peripheral metabotropic glutamate receptor 5 mediates mechanical hypersensitivity in craniofacial muscle via protein kinase C dependent mechanisms. Neurosci. 2007; 146(1):375–383.

Likar R, Koppert W, Blatnig H, Chiari F, Sittl R, Stein C, Schafer M. Efficacy of peripheral morphine analgesia in inflamed, non-inflamed and perineural tissue of dental surgery patients. J Pain Symptom Manage. 2001; 21:330–337. [PubMed: 11312048]

Modi M, Rastogi S, Kumar A. Buprenorphine with bupivacaine for intraoral nerve blocks to provide postoperative analgesia in outpatients after minor oral surgery. J Oral Maxillofac. Surg. 2009; 67:2571–2576. [PubMed: 19925973]

Niu K, Saloman JL, Zhang Y, Ro JY. Sex differences in the contribution of ATP-sensitive K+ channels in trigeminal ganglia under an acute muscle pain condition. Neurosci. 2011; 180:344–352.

North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. Proc Natl Acad Sci USA. 1987; 84:5487–5491. [PubMed: 2440052]

Núñez S, Lee JS, Zhang Y, Bai G, Ro JY. Role of peripheral mu-opioid receptors in inflammatory orofacial muscle pain. Neuroscience. 2007; 146:1346–1354. [PubMed: 17379421]

Obara I, Parkitna JR, Korostynski M, Makuch W, Kaminska D, Przewlocka B, Przewlocki R. Local peripheral opioid effects and expression of opioid genes in the spinal cord and dorsal root ganglia in neuropathic and inflammatory pain. Pain. 2009; 141:283–291. [PubMed: 19147290]

Pacheco DF, Duarte ID. Delta-opioid receptor agonist SNC80 induces peripheral antinociception via activation of ATP-sensitive K+ channels. Eur J Pharmacol. 2005; 512:23–28. [PubMed: 15814086]

Pacheco DF, Reis GM, Francischi JN, Castro MS, Perez AC, Duarte ID. Delta-opioid receptor agonist SNC80 elicits peripheral antinociception via delta(1) and delta(2) receptors and activation of the l-arginine/nitric oxide/cyclic GMP pathway. Life Sci. 2005; 78:54–60. [PubMed: 16135369]

Patwardhan AM, Diogenes A, Berg KA, Fehrenbacher JC, Clarke WP, Akopian AN, Hargreaves KM. PAR-2 agonists activate trigeminal nociceptors and induce functional competence in the delta opioid receptor. Pain. 2006; 125:114–124. [PubMed: 16781076]

Pena-dos-Santos DR, Severino FP, Pereira SA, Rodrigues DB, Cunha FQ, Vieira SM, Napimoga MH, Clemente-Napimoga JT. Activation of peripheral kappa/delta opioid receptors mediates 15-deoxy-(Delta12,14)-prostaglandin J2 induced-antinociception in rat temporomandibular joint. Neuroscience. 2009; 163:1211–1219. [PubMed: 19647045]

Picolo G, Cassola AC, Cury Y. Activation of peripheral ATP-sensitive K+ channels mediates the antinociceptive effect of crotalus durissus terrificus snake venom. Eur J Pharmacol. 2003; 469:57–64. [PubMed: 12782185]

Porreca F, Takemori AE, Sultana M, Portoghese PS, Bowen WD, Mosberg HI. Modulation of mu-mediated antinociception in the mouse involves opioid delta-2 receptors. J Pharmacol Exp Ther. 1992; 263:147–152. [PubMed: 1328602]

Portoghese PS, Sultana M, Takemori AE. Naltrindole a highly selective and potent non-peptide δ opioid receptor antagonist. Eur J Pharmacol. 1988; 146:185–186. [PubMed: 2832195]

Ro JY, Capra N, Masri R. Development of a behavioral assessment of craniofacial muscle pain in lightly anesthetized rats. Pain. 2003; 104:179–185. [PubMed: 12855327]

Ro JY, Lee J, Capra NF, Zhang Y. Role of soluble guanylate cyclase in the trigeminal subnucleus caudalis in capsaicin-induced muscle hypersensitivity. Brain Res. 2007; 1184:141–148. [PubMed: 17980861]

Ro JY, Lee JS, Zhang Y. Activation of TRPV1 and TRPA1 leads to muscle nociception and mechanical hyperalgesia. Pain. 2009; 144:270–277. [PubMed: 19464796]

Rodrigues AR, Duarte ID. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K(+) channels. Br J Pharmacol. 2000; 129:110–114. [PubMed: 10694209]

Rorarius M, Suominen P, Baer G, Pajunen P, Tuimala R, Laippala P. Peripherally administered sufentanil inhibits pain perception after postpartum tubal ligation. Pain. 1999; 79:83–88. [PubMed: 9928780]

Rowan MP, Ruparel NB, Patwardhan AM, Berg KA, Clarke WP, Hargreaves KM. Peripheral delta opioid receptors require priming for functional competence in vivo. Eur J Pharmacol. 2009; 602:283–287. [PubMed: 19063879]

Sachs D, Cunha FQ, Ferreira SH. Peripheral analgesic blockade of hypernociception: Activation of arginine/NO/cGMP/protein kinase G/ATP-sensitive K+ channel pathway. Proc Natl Acad Sci USA. 2004; 101:3680–3685. [PubMed: 14990791]

Sánchez EM, Bagües A, Martín MI. Contributions of peripheral and central opioid receptors to antinociception in rat muscle pain models. Pharmacol Biochem Behav. 2010; 96(4):488–495. [PubMed: 20637793]

Sheldon R, Riviere PJ, Malarchik ME, Moseberg HI, Burks TF, Porreca F. Opioid regulation of mucosal ion transport in the mouse isolated jejunum. J Pharmacol Exp Ther. 1990; 253:144–151. [PubMed: 2329501]

Shinoda K, Hruby VJ, Porreca F. Antihyperalgesic effects of loperamide in a model of rat neuropathic pain are mediated by peripheral delta-opioid receptors. Neurosci Lett. 2007; 411:143–146. [PubMed: 17110034]

Standifer KM, Pasternak GW. G Proteins and Opioid Receptor-Mediated Signaling. Cell Signa. 1997; 9(3/4):237–248.

Stein C, Millan MJ, Shippenberg TS, Peter K, Herz A. Peripheral opioid receptors mediating antinociception in inflammation. evidence for involvement of mu, delta and kappa receptors. J Pharmacol Exp Ther. 1989; 248:1269–1275. [PubMed: 2539460]

Stein C, Schafer M, Machelska H. Attacking pain at its source: New perspectives on opioids. Nat Med. 2003; 9:1003–1008. [PubMed: 12894165]

Wang HB, Zhao B, Zhong YQ, Li KC, Li ZY, Wang Q, Lu YJ, Zhang ZN, He SQ, Zheng HC, Sheng-Xi, Hökfelt GM, Bao L, Zhang X. Coexpression of δ- and μ-opioid receptors in nociceptive sensory neurons. PNAS. 2010; 107:13117–13122. [PubMed: 20615975]

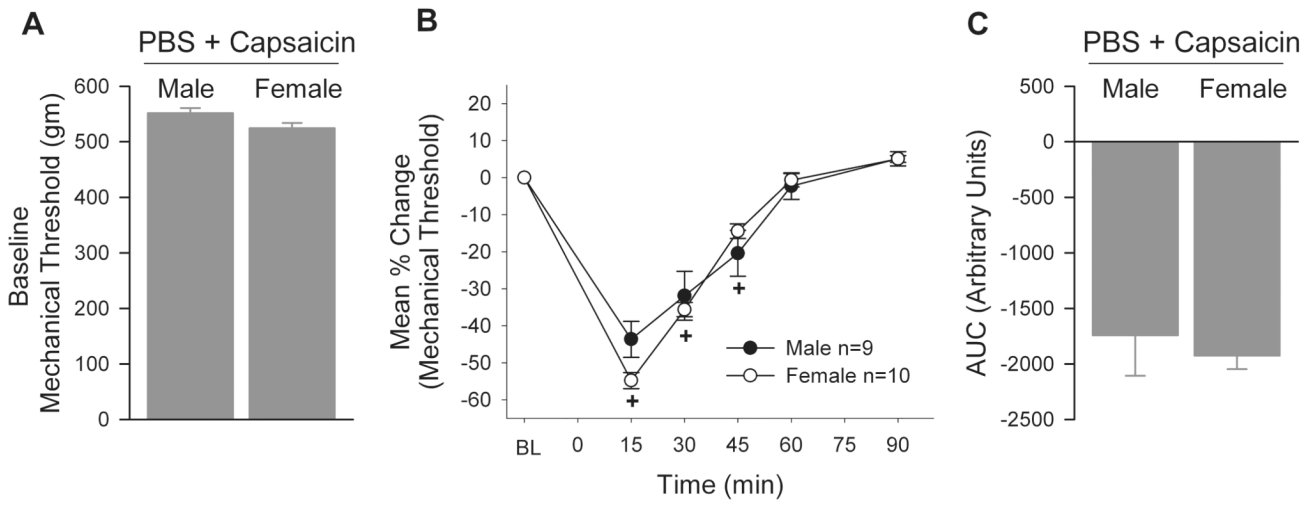

Figure 1.

(A) Baseline mechanical thresholds of the masseter muscle were assessed in male and female rats. (B,C) Capsaicin induced a reduction in the mechanical threshold of both male and female rats in a time-dependent manner. The line graph shows the time course and bar graph shows the overall magnitude of responses. + denotes a significant time effect.

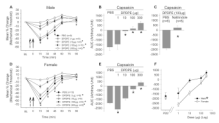

Figure 2.

(A,B) Local DPDPE pretreatment dose-dependently attenuated the capsaicin-induced masseter hypersensitivity in male rats. The line graph shows the time course and the bar graph the overall magnitude of responses. +, * denote significant time and drug effects, respectively, in this and subsequent figures. (C) The effect of DPDPE was prevented by pretreatment with Naltrindole (100μg). (D,E) The line graph and bar graph show female responses to the same doses of DPDPE. (F) Analysis of dose responses revealed a significant dose effect (+) as well as a significant sex effect (*). BL: baseline; dashed arrow: DPDE or PBS injection; solid arrow: capsaicin injection

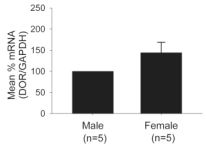

Figure 3.

Real-Time RT-PCR analysis of DOR mRNA in TG from naïve male and female rats revealed no significant difference (n=5 for each group). Female data were normalized to male which were set at 100%. Data are presented as mean ±SE.

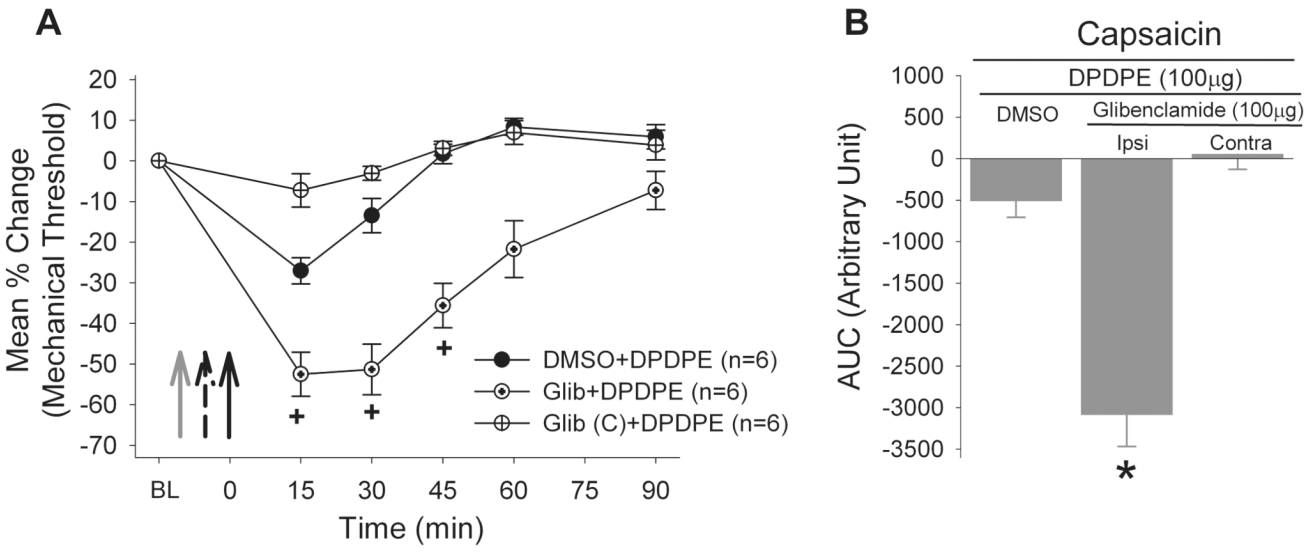

Figure 4.

In male rats, the effect of DPDPE on capsaicin-induced masseter hypersensitivity was prevented when glibenclamide, a KATP antagonist, was pre-administered into the same muscle. The same dose of glibenclamide given in the contralateral masseter did not block the DPDPE effect indicating glibenclamide produced its effect via antagonizing local KATPs (Ipsi-ipsilateral, C- and Contra-contralateral). BL: baseline; gray arrow: DMSO or glibenclamide injection; dashed arrow: DPDPE injection; solid arrow: capsaicin injection