# Sex Differences in Opioid-Induced Enhancement of Contact Hypersensitivity

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**Previous research has demonstrated that, in male rats, the magnitude of contact hypersensitivity (CHS) can be enhanced by morphine treatment. The present experiments test the hypothesis that the μ-opioids morphine, etorphine, and buprenorphine would produce significant sex differences in the magnitude of 2,4-dinitrofluorobenzene-induced CHS. During tests conducted over a 192-h period, morphine, etorphine, and buprenorphine administered before elicitation of CHS on the external surface of the ear (pinna) potentiated the CHS response, and the magnitude of this enhancement was significantly greater in females than males. By contrast, morphine had no effect on croton oil-induced irritant contact dermatitis, indicating that morphine's effects on CHS do not generalize to immunologically nonspecific forms of contact dermatitis. Activation of brain μ-opioid receptors is responsible for the effects of morphine on CHS, because intracerebroventricular treatment with the μ-opioid receptor antagonist β-funaltrexamine blocked morphine potentiation of CHS in females and males. The sex differences in morphine potentiation of CHS appear to be a result of the gonadal hormonal milieu, because castration enhanced the CHS response following vehicle and morphine treatment, whereas ovariectomy significantly attenuated the enhancement of CHS by morphine. Because ovariectomy had no effect on the CHS response following vehicle treatment, the presence of female gonadal hormones may underlie the sex differences in morphine potentiation of CHS in gonadally intact animals. Overall, these results support an increased sensitivity to the modulatory effects of opioids on the CHS response in females that depends on the interaction between gonadal hormones and the central μ-opioid system.** ***Key words: contact dermatitis/morphine/etorphine/buprenorphine. J Invest Dermatol 121:1053–1059, 2003***

Contact hypersensitivity (CHS) is a cutaneous form of delayed-type hypersensitivity that results in inflammation following multiple exposures to a haptenized chemical antigen. This prevalent condition accounts for 20% to 25% of all cases of contact dermatitis (Marks, 2002). Following initial exposure to the hapten (sensitization), proinflammatory cytokines such as interleukin-1β and tumor necrosis factor-α are rapidly produced (Enk and Katz, 1992) that activate Langerhans cells and stimulate antigen processing. In addition, these cytokines promote Langerhans cell migration to the skin-draining lymph nodes, where they present antigen to naïve T cells that differentiate into antigen-specific memory T cells (for a review see Grabbe and Schwarz, 1998). After subsequent exposure to the antigen (elicitation), a host of proinflammatory cytokines and chemokines are produced by local keratinocytes and monocytes at the elicitation site along with upregulation of major histocompatibility complex and adhesion molecules resulting in infiltration of other cells such as neutrophils and T lymphocytes (Grabbe and Schwarz, 1998). Like other delayed-type hypersensitivity responses, CHS has been classically viewed as Th1-mediated (Cher and Mosmann, 1987); however, recent evidence suggests the key involvement of both Th2 and CD8+ cells (Bour *et al*, 1995; Xu *et al*, 1996). Therefore, CHS is an immunologically complex, oligoclonal response to epicutaneously applied haptens that results in swelling, redness, and pain at the elicitation site.

Recent studies indicate that the magnitude of the CHS response can be dramatically altered in rodents by pharmacologic manipulations. Work in our laboratory has demonstrated that morphine given before elicitation of CHS (but not sensitization with antigen) significantly increases pinna swelling in a model of 2,4-dinitrofluorobenzene (DNFB)-induced CHS in male rats (Nelson *et al*, 1999). Follow-up work demonstrated the involvement of proinflammatory mediators, brain opioid receptors, and neurokinin-1 receptor activation in the ear in mediating the effects of morphine on the CHS response (Nelson and Lysle, 2001a, b). In addition to enhancing the CHS response, morphine has many other immunomodulatory effects including degranulating cutaneous mast cells (Marone *et al*, 1993; Di Bello *et al*, 1998), altering T and B lymphocyte proliferation (Lysle *et al*, 1993), and increasing nitric oxide production (Fecho *et al*, 1994). Taken together, these results demonstrate that morphine is an immunomodulatory drug that acts via central opioid receptors to potentiate CHS in male rats by increasing levels of various proinflammatory mediators at the site of CHS elicitation.

To date, the investigations of the effects of morphine on the CHS response have focused on male animals. Given identified sex differences in opioid-induced antinociception (Cicero *et al*,

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Abbreviations: CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene; ICD, irritant contact dermatitis.

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1996; Cook *et al*, 2000; Sarton *et al*, 2000), physical dependence (Cicero *et al*, 2002), and emesis (Zun *et al*, 2002), sex is likely to be a crucial determinant of the magnitude of opioid modulation of CHS. Furthermore, there is evidence suggesting that gonadal hormones may mediate these sex differences because gonadectomy has been shown to attenuate or reverse observed sex differences in opioid antinociception (Terner *et al*, 2002). Therefore, this investigation tested the hypotheses that morphine and other μ-opioids will produce sex differences in the CHS response and that these sex differences will depend on gonadal hormone milieu.

In these experiments, the effect of several selective μ-opioids on the magnitude of DNFB-induced CHS was assessed in male and female rats. To that end, animals were administered vehicle or selected doses of the μ-opioids morphine, etorphine, or buprenorphine before CHS elicitation with DNFB. Morphine and buprenorphine are clinically used analgesics with high and low efficacy at the μ receptor, respectively, whereas etorphine is a relatively short-acting high-efficacy agonist at the μ-opioid receptor. Buprenorphine (Subutex, Schering-Plough Corp., Kenilworth, NJ) has also recently been approved by the US Food and Drug Administration as a treatment for heroin dependence that can be prescribed by primary care physicians. These drugs were chosen to test the generalizability of observed sex differences to a range of clinically used opioids and establish the immunomodulatory effects of buprenorphine and etorphine in the skin. Subsequently, experiments were conducted to assess potential mechanisms underlying the observed effects, including whether: (1) morphine's enhancement of the CHS response generalizes to the immunologically nonspecific cutaneous inflammatory response irritant contact dermatitis (ICD); (2) morphine's potentiation of the CHS response in both males and females depends on activation of brain and/or peripheral μ-opioid receptors; and (3) sex differences in the effects of morphine on the CHS response depends on the gonadal hormone milieu.

## MATERIALS AND METHODS

**Animals** Pathogen-free adult Fischer rats (3–6 mo of age) obtained from Charles River (Raleigh, NC) were used in these investigations. Animals were individually housed and kept on a 12:12-h light cycle with lights off at 3 p.m. A combination of drug-naïve animals and animals who had previously received brief exposure to opioids were used in the concentration–response studies. All animals previously exposed to opioids were given a drug-free period of at least 3 wk before receiving any treatment in the present experiments. Fischer rats gonadectomized by the vendor (Charles River) at approximately 60 d old were also used following a 4-wk period after surgery to dissipate remaining circulating gonadal hormones (Cicero *et al*, 1996). Postmortem examinations of gonadectomized animals were also conducted to confirm the absence of ovaries or testes. All procedures employed in these studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina at Chapel Hill.

**Drugs** Morphine sulfate, etorphine hydrochloride, buprenorphine hydrochloride, and β-funaltrexamine hydrochloride were provided by the National Institute on Drug Abuse (NIDA, Bethesda, MD). All drugs, except where noted, were dissolved in endotoxin-free sterile water and delivered by subcutaneous injection to a site on the lateral lower abdomen in a final volume of 1 mL per kg.

**Induction of CHS** Sensitization and elicitation of CHS with DNFB (Sigma, St. Louis, MO) was performed as previously described (Nelson *et al*, 1999). Briefly, animals were sensitized with 100 μL of 1% DNFB (in a 4:1 mixture of acetone and olive oil) applied to the shaved abdomen on days 1 and 2 of the experiment. On day 6, the animals were administered selected doses of morphine (2.5, 7.5, or 15 mg/kg), buprenorphine (0.01, 0.1, or 1.0 mg/kg), etorphine (0.001, 0.005, or 0.01 mg/kg), or vehicle alone (sterile water) by subcutaneous injection. One hour later, 25 μL of a 0.5% DNFB solution was applied to the treatment ear (right pinna) to elicit the CHS response. As a control, an equivalent volume of vehicle alone was applied to the opposing ear (left pinna). The course of the inflammatory response was measured at 12 and 24 h after CHS elicitation and every 24 h thereafter to 192 h after elicitation.

**Induction of ICD** Induction of ICD was performed as previously described (Nelson *et al*, 1999). Animals were treated with 15 mg per kg morphine or equivalent volume of sterile water 1 h before elicitation of ICD on the right pinna by the application of 50 μL of 10% croton oil (Sigma) in a 4:1 acetone and olive oil vehicle. As a control, the left pinna was treated with an equivalent volume of vehicle alone. Right and left pinna thicknesses were measured at 1, 2, 4, 6, 8, 12, and 24 h after ICD elicitation.

**Central μ-opioid receptor antagonism** To assess the involvement of central μ-opioid receptors in morphine enhancement of the CHS response in female and male rats, animals were stereotaxically implanted with unilateral 22-gauge cannulae (Plastics One, Roanoke, VA) directed at the left lateral ventricle before sensitization with DNFB on day 1 of the experiment (Nelson and Lysle, 2001a). Sensitization and elicitation of CHS were performed as described above. On the day before CHS elicitation (day 5), animals received intracerebroventricular infusions of 20 μg of the irreversible μ-opioid receptor β-funaltrexamine HCl dissolved in 7.5 μL of 0.9% physiologic saline or saline alone. Twenty-four hours after intracerebroventricular infusions, animals received injections of either 15 mg per kg morphine or vehicle, and CHS was elicited 1 h later. Pinna thickness was measured 24, 48, and 72 h after CHS elicitation.

**Measurement and data analysis** Swelling was quantified by measuring pinna thickness using a digimatic caliper (Mitutoyo, Kawasaki, Japan). At each time point both the right (DNFB- or croton oil-treated) and the left (control) pinnae were measured twice, and the means of each pinna thickness were calculated for statistical analysis. If measurements differed by more than 0.05 mm, one additional measurement was taken and the mean was calculated from the three measurements for use in the analyses. This additional measurement was required in approximately 25% of all cases throughout the experiments described herein. All measurements were then compared to a baseline pinna thickness taken on the day before elicitation of inflammation to give a percentage of change from baseline value. This metric was chosen as it allows standardization to control for baseline differences in ear thickness. The specific formula used was (measurement − baseline)/baseline × 100.

All data were analyzed using ANOVA. Where appropriate, standard polynomial contrasts and Fisher's LSD *post hoc* analyses were conducted to probe main and interaction effects (Kirk, 1982). Statistical significance for all analyses was set at $p < 0.05$.

## RESULTS

**CHS response: vehicle treatment** Pilot data indicated that the concentration of DNFB used to elicit CHS on the pinna (0.5%) produced no inflammation in unsensitized male or female rats (data not shown). Nevertheless, exposure to 0.5% DNFB in animals sensitized with 1% DNFB produced pinna inflammation under drug-free conditions that reached a peak of 30% to 40% above baseline at approximately 72 h after CHS elicitation and declined thereafter toward baseline values (**Fig 1**). Consistent with our hypotheses, there was no significant sex difference in the magnitude of the inflammatory response following vehicle treatment (**Table I**). Therefore, our experimental protocol produced time-dependent inflammation in control animals that was specific to sensitized animals and did not differ between males and females.

**Morphine: intact animals** Morphine ($F_{3,40} = 25.76$, $p < 0.001$, **Fig 1**) significantly potentiated the inflammatory effects of DNFB in a dose-dependent manner as evidenced by increased pinna swelling for both males and females compared to vehicle controls. There was also a significant effect of sex ($F_{1,40} = 26.33$, $p < 0.001$), indicating that the pinnae of females swelled to a significantly greater extent than those of males. Importantly, a significant linear interaction between morphine and sex ($F_{1,40} = 5.20$, $p = 0.028$) was also observed, demonstrating that the magnitude of the difference between females and males became greater with increasing doses of morphine. Supporting

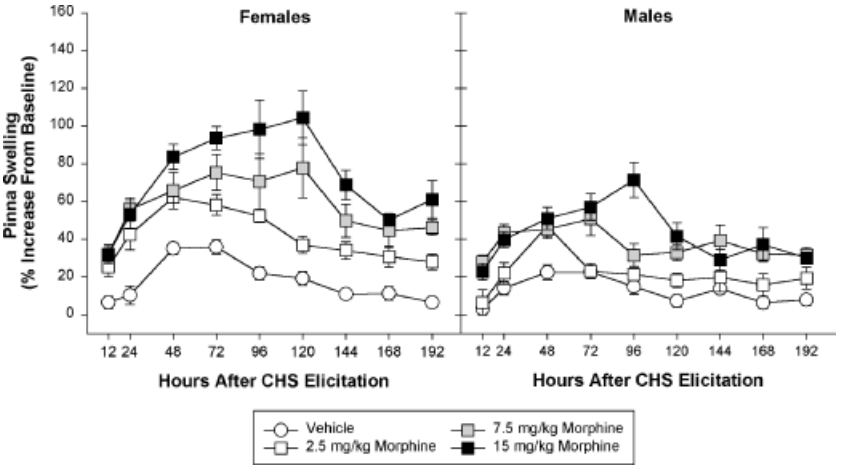


**Figure 1. Female Fischer rats display significantly greater morphine-induced potentiation of the CHS response than male rats.** Pinna measurements were taken from 12 to 192 h after CHS elicitation, and data represent the percentage of change in pinna thickness from a baseline value taken before DNFB application (mean ± SEM, n = 6). Whereas there was no significant effect of sex on the magnitude of inflammation following vehicle treatment ($p = 0.256$), females displayed increasingly greater inflammation than males with increasing doses of morphine ($p = 0.028$).

**Table I. Post-Hoc Comparisons (Fisher's LSD) of Sex Differences in the CHS Response Following Vehicle or Opioid Administration**

| Drug | Dose (mg/kg) | Mean Difference (Females–Males) | t | df | p |
|---|---|---|---|---|---|
| Vehicle (Sterile Water) | N/A | 5.00 | 1.20 | 10 | 0.256 |
| Morphine | 2.5 | 19.73 | 2.71 | 40 | 0.01 |
| | 7.5 | 20.33 | 2.80 | | 0.008 |
| | 15 | 29.49 | 4.06 | | 0.001 |
| Etorphine | 0.001 | 6.57 | 0.75 | 40 | 0.457 |
| | 0.05 | 17.53 | 2.63 | | 0.012 |
| | 0.01 | 22.30 | 3.35 | | 0.002 |
| Buprenorphine | 0.01 | 5.31 | 0.76 | 40 | 0.449 |
| | 0.1 | 14.30 | 2.18 | | 0.035 |
| | 1.0 | 28.97 | 4.42 | | 0.001 |

this finding, *post hoc* analyses of the sex difference at each dose level revealed that, whereas pinna thickness in males and females did not differ following vehicle administration, at each dose of morphine tested females displayed significantly greater inflammation than males (**Table I**). It is also interesting to note that whereas treatment with 2.5 mg per kg morphine produced significant enhancement of inflammation in females compared to saline ($t(40) = 3.25$, $p = 0.02$), this dose of morphine produced no significant enhancement of inflammation over saline treatment in males ($t(40) = 1.23$, $p = 0.23$). Morphine did not cause generalized cutaneous inflammation independent of DNFB treatment, because there was no significant treatment effect on the thickness of the vehicle-treated left ear ($F_{3,40} = 0.252$, $p = 0.86$). Therefore, morphine produces greater peak inflammatory effects and is more potent in potentiating DNFB-induced CHS in females than males.

To control for differences in baseline ear thickness between male and female animals, analyses were also performed using raw difference from baseline scores (i.e., pinna thickness – baseline thickness). Using this approach, the pattern of results was similar to results obtained using percentage of change scores (data not shown), indicating that sex differences in response to morphine treatment were independent of differences in baseline pinna thickness.

**Etorphine: intact animals** Etorphine significantly enhanced pinna inflammation in both female and male rats ($F_{3,40} = 39.66$, $p < 0.001$, **Fig 2*A***), and females displayed significantly greater inflammation than males across all treatment conditions ($F_{1,40} = 14.93$, $p < 0.001$). Nevertheless, as with morphine treatment, a significant linear interaction between etorphine treatment and sex was observed ($F_{1,40} = 4.47$, $p = 0.041$), indicating that the magnitude of the sex difference increased with greater doses of etorphine. *Post hoc* comparisons revealed that females displayed significantly greater inflammation than males following treatment with 0.01 and 0.005 mg per kg etorphine, but not following vehicle or the 0.001 mg per kg dose (**Table I**). Overall, the results obtained with etorphine are similar to those obtained with morphine in that etorphine produced dose-dependent enhancement of the CHS response in males and females, and etorphine was significantly more potent in producing proinflammatory effects in females than males.

**Buprenorphine: intact animals** The low-efficacy μ-opioid buprenorphine significantly enhanced DNFB-induced pinna swelling ($F_{3,40} = 11.13$, $p < 0.001$, **Fig 2*B***), and consistent with other drugs tested, females displayed significantly greater inflammation than males ($F_{1,40} = 16.74$, $p < 0.001$). The magnitude of the sex difference grew with increasing doses of buprenorphine, as evidenced by a linear buprenorphine by sex interaction ($F_{1,40} = 7.631$, $p = 0.009$). *Post hoc* comparisons indicated that a significant sex difference was present following treatment with 0.1 and 1.0 mg buprenorphine, but not following vehicle or 0.01 mg per kg buprenorphine (**Table I**). These results demonstrate that like the other μ-opioids tested, buprenorphine produced significantly greater enhancement of DNFB-induced inflammation in females than males.

**Morphine: effects on ICD** To determine the specificity of sex differences in morphine potentiation of CHS, the effect of 15 mg per kg morphine given 1 h before application of 10% croton oil was assessed. Morphine did not significantly alter the ICD response ($F_{1,19} = 0.77$, $p = 0.39$, **Fig 3**), nor was there a significant sex difference ($F_{1,19} = 0.344$, $p = 0.66$) or morphine by sex interaction ($F_{1,19} = 0.01$, $p = 0.92$). Therefore, morphine's sexually dimorphic enhancing effects on CHS do not generalize to ICD, a common immunologically nonspecific form of cutaneous inflammation.

**Central μ-opioid receptor antagonism** Following intracerebroventricular pretreatment with 20 μg of the irreversible μ-opioid antagonist β-funaltrexamine, both male and female rats displayed complete antagonism of morphine (15 mg/kg) potentiation of CHS ($F_{1,37} = 29.08$, $p < 0.001$, **Fig 4**). These data demonstrate that morphine acts via μ-opioid receptors in the central nervous system and not those expressed on immunocytes to enhance CHS in male and female rats. There was no sex difference in the magnitude of antagonism ($F_{1,37} = 0.60$, $p = 0.44$), indicating that activation of brain μ-opioid receptors is

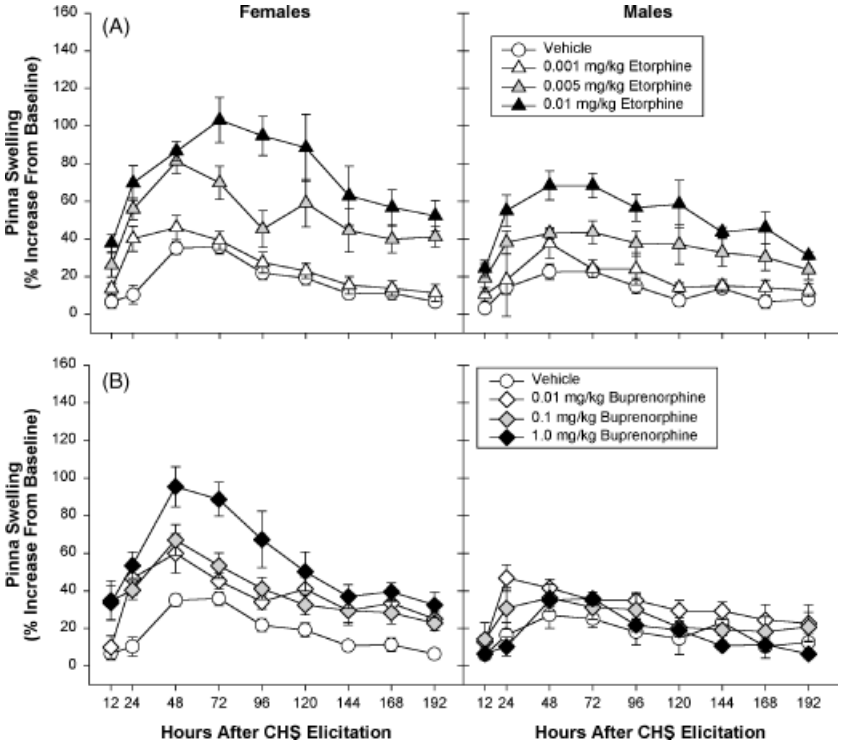


**Figure 2. Female Fischer rats display significantly greater (*A*) etorphine- and (*B*) buprenorphine-induced potentiation of the CHS response than male rats.** Pinna measurements were taken from 12 to 192 h after CHS elicitation, and data represent the percentage of change in pinna thickness from a baseline value taken before DNFB application (mean ± SEM, n = 6). Whereas no sex difference was present following vehicle treatment ($p > 0.05$), both etorphine ($p = 0.041$) and buprenorphine ($p = 0.009$) produced concentration dependently greater inflammation in females than males.

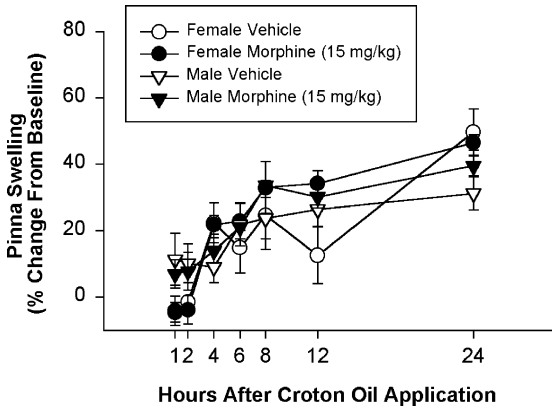


**Figure 3. Morphine (15 mg/kg) does not alter the magnitude of croton oil-induced ICD in female or male Fischer rats.** Pinna measurements were taken from 1 to 24 h after croton oil application, and data represent the percentage of change in pinna thickness from a from a baseline value taken prior to croton oil application (mean ± SEM, n = 5–6). The magnitude of inflammation was unaffected by morphine treatment ($p = 0.39$) nor was there a significant sex difference in the inflammation observed ($p = 0.66$).

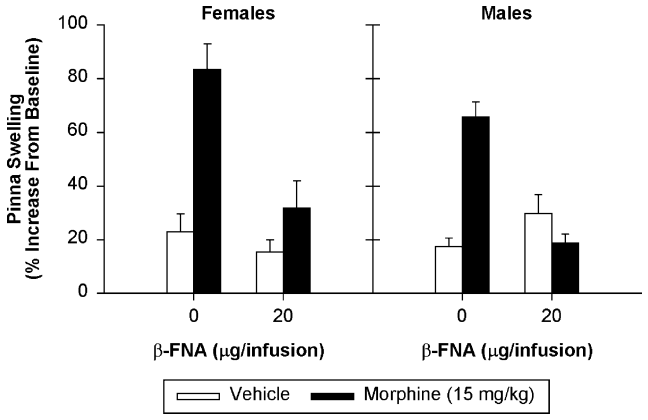


**Figure 4. Brain μ-opioid receptors are responsible for morphine potentiation of CHS in male and female rats.** Animals received infusions of the irreversible μ-opioid antagonist β-funaltrexamine (20 μg) or saline 24 h before treatment with vehicle or 15 mg per kg morphine (given 1 h before CHS elicitation). Data represent the percentage of change in pinna thickness from a baseline value taken the day before DNFB application and are pooled from measurements taken at 24, 48, and 72 h after CHS elicitation (mean ± SEM, n = 5–7). β-Funaltrexamine treatment significantly antagonized morphine potentiation of the CHS response in males and females ($p < 0.001$) but had no effect on the magnitude of inflammation following vehicle treatment ($p = 0.67$).

responsible for the effects of morphine on CHS in both males and females. Importantly, β-funaltrexamine had no effect on the CHS response following vehicle treatment ($t(20) = 0.439$, $p = 0.67$), demonstrating that functional brain μ-opioid receptors are not required for the elicitation phase of CHS.

### Gonadectomy experiment

*Females* In ovariectomized and sham-operated animals, there was a significant effect of both morphine treatment ($F_{2,35} = 30.31$, $p < 0.001$) and hormonal status ($F_{1,35} = 13.31$, $p = 0.001$). There was also a significant linear interaction between morphine treatment and hormonal status ($F_{1,35} = 13.72$, $p = 0.001$), indicating that whereas there was no significant difference in the magnitude of inflammation between ovariectomized and sham-operated females following vehicle treatment, significant differences emerged following administration of increasing doses of morphine (**Fig 5*A***). These results indicate a crucial role of female gonadal hormones in potentiating morphine's effects on the CHS response, but not the normal CHS response manifest in the absence of drug treatment.

*Males* In this experiment, morphine treatment ($F_{2,36} = 65.40$, $p < 0.001$) enhanced the CHS response in castrated and sham-operated males (**Fig 5*B***). Additionally, there was a significant effect

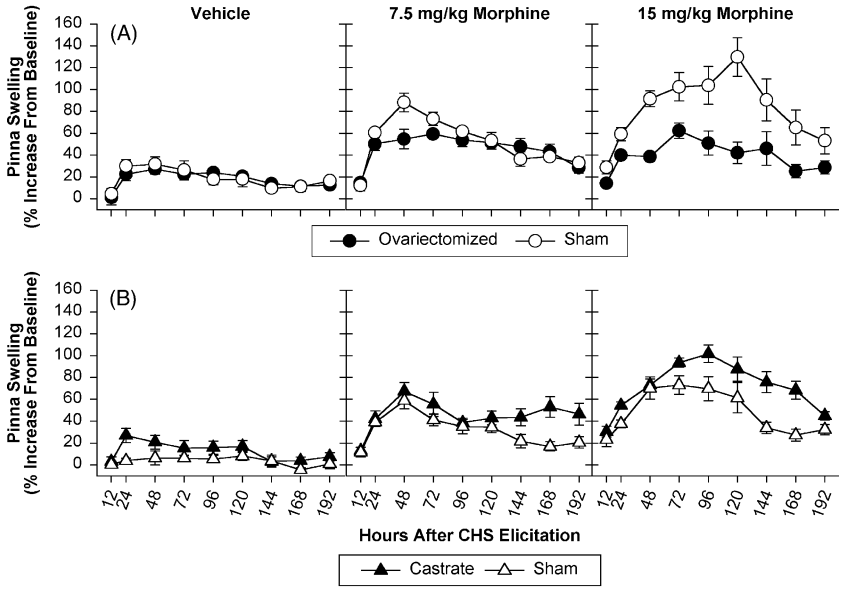


**Figure 5. Castration and ovariectomy produce opposing effects on morphine enhancement of CHS.** (*A*) Ovariectomy attenuates morphine potentiation of CHS, as evidenced by a significant interaction between hormonal status and morphine treatment ($p = 0.001$). (*B*) Castration enhances CHS following vehicle and morphine treatment, as evidenced by a significant effect of castration ($p < 0.001$) and morphine treatment ($p < 0.001$), but no morphine by castration interaction ($p = 0.34$). Pinna measurements were taken from 12 to 192 h after CHS elicitation, and data represent the percentage of change in pinna thickness from a baseline measurement taken before DNFB application (mean ± SEM, $n = 5$).

of hormonal status ($F_{1,36} = 17.07$, $p < 0.001$) but not a morphine by hormonal status interaction ($F_{2,36} = 1.12$, $p = 0.337$). Therefore, castration produced an increase in the magnitude of the CHS response in males following both vehicle and morphine treatment. This finding suggests that the presence of male gonadal hormones suppresses the CHS response. Nevertheless, the presence of male gonadal hormones likely makes a limited contribution to the sex differences observed in gonadally intact animals following morphine treatment, because hormone status and morphine treatment do not interact to produce differential inflammatory responses in male animals.

## DISCUSSION

This report demonstrates sex differences in opioid modulation of CHS and that gonadal hormones appear to play a critical role in mediating this effect. These experiments extend previous research showing that morphine enhances the CHS response in male rats (Nelson *et al*, 1999; Nelson and Lysle, 2001a) by demonstrating that μ-opioids with diverse pharmacologic properties produce significantly greater DNFB-induced cutaneous inflammation in females than males. By contrast, sex differences are not present following vehicle treatment, indicating that activation of the opioid system is required for the emergence of sex differences. The present data also establish the potential of the clinically used analgesic buprenorphine and the high-efficacy μ-opioid etorphine to alter cutaneous immune responses. In addition, these studies provide important insights into the mechanisms underlying morphine's potentiation of CHS in that that morphine's enhancement of the CHS response in both male and female rats does not generalize to croton oil-induced ICD and is mediated exclusively by activation of brain μ-opioid receptors. Finally, evidence that castration enhances whereas ovariectomy suppresses morphine enhancement of CHS suggests that the influence of gonadal hormone milieu is responsible for the sex differences observed in intact animals.

While it is clear that the magnitude of the CHS response is significantly greater in females than males following treatment with μ-opioid agonists at the time of CHS elicitation, it remains to be established which determinants of CHS are differentially altered in males and females in the presence of opioids. Previous research in male rats demonstrated that mRNA levels of the proinflammatory mediators inducible nitric oxide synthase, interleukin-6, and interferon-γ were upregulated in the DNFB-treated pinnae of morphine compared to saline-treated animals at various time periods in the first 24 h after elicitation of CHS (Nelson and Lysle, 2001b). In contrast, in the same investigations there was no treatment-related change in the anti-inflammatory Th2-derived cytokine interleukin-10 over the same time course. Therefore, it is possible that morphine preferentially potentiates the expression in fermales of either proinflammatory mediators such as those described above or other cytokines known to be involved in the expression of contact hypersensitivity such as interleukin-1β or tumor necrosis factor-α (Kondo *et al*, 1995; McHale *et al*, 1999). To address these possibilities, a time–course examination of relevant monocyte and T lymphocyte-derived cytokine levels in the pinna and their relation to sex and morphine treatment is required.

In addition to cytokine expression, several other immunologic and nonimmunologic mechanisms may prove to account for the observed sex differences. For instance, sex differences may exist in antigen processing or presentation, expression of cell adhesion molecules (e.g., selectins), or anti-DNP antibody production (Ray *et al*, 1983) following morphine treatment. Data from our laboratory have also demonstrated that local pretreatment of the pinna with the neurokinin-1 receptor antagonist WIN51,708 antagonizes morphine potentiation of the CHS response in male rats (Nelson and Lysle, 2001a). The endogenous neurokinin-1 ligand substance P appears to promote the induction of CHS (Niizeki *et al*, 1999) and substance P agonists cause vasodilation and plasma extravasation from cutaneous vasculature by releasing histamine from cutaneous mast cells (Holzer, 1992). Therefore, sex differences in morphine enhancement of the CHS response may be due to differential modulation of the immunologic determinants or vasoactive mediators involved in the induction of CHS.

The present experiments also provide evidence suggesting putative mechanisms underlying sex differences in morphine enhancement of the CHS response. As evidenced by the failure of morphine to promote croton oil-induced irritation of the pinna, these data suggest that our findings do not generalize to other

forms of contact dermatitis. ICD accounts for 80% of cases of human contact dermatitis (Marks, 2002), does not require prior exposure to the irritant, and is believed to result from release of proinflammatory cytokines from T lymphocytes and keratinocytes owing to acute tissue injury (Baadsgaard and Wang, 1991). These data support previous research demonstrating that morphine does not alter the course of ICD in male Lewis rats (Nelson *et al*, 1999) and suggest that the greater enhancement of CHS in females by morphine occurs via interaction with antigen-specific effector mechanisms. Another important finding concerning mechanisms of morphine potentiation of CHS is that these effects occur owing to activation of brain μ-opioid receptors. Because morphine has been shown to alter immune responses via interaction with opioid receptors located in the brain (Mellon and Bayer, 1998) and those expressed on immunocytes (Eisenstein and Hilburger, 1998), it was of critical importance to determine the anatomic distribution and specific subtype of opioid receptor responsible for these effects. Therefore, future studies will focus on potential sex differences in central μ-opioid function that might account for sex differences in morphine's effects on CHS.

Our results demonstrate that ovariectomized females treated with morphine, but not vehicle, display attenuated CHS responses compared to intact females. In contrast, castration significantly enhances both the basal and the morphine-modulated CHS response in males. Together, these findings suggest that males and females have unique hormonal milieus that contribute differentially to the expression of CHS following morphine or vehicle treatment. Nevertheless, unlike the enhancing effect of castration in male animals which was relatively equivalent following both vehicle and morphine treatment, the dramatic inhibitory effect of ovariectomy on the CHS response is only apparent following morphine treatment. Therefore, the presence of female gonadal hormones is likely largely responsible for the sex differences in opioid-potentiated inflammation in intact animals. This pattern of opposing effects of female and male gonadal hormones on immune parameters (Leposavic *et al*, 1996; Travi *et al*, 2002) and responses to opioids (Terner *et al*, 2002) has been well established in other investigations. Nevertheless, the unique feature of the current investigations is that the effects of gonadectomy, and in particular ovariectomy, on CHS emerged in the presence of morphine treatment. Although the goal of the present studies was not to determine the hormonal locus of these effects, gonadectomy produces a marked suppression of circulating prolactin levels (Shaar *et al*, 1975), an anterior pituitary hormone whose plasma levels are increased by morphine (Baumann *et al*, 2000). Because prolactin produces proinflammatory effects by promoting a Th1 cytokine profile, it will be important to consider the potential role of this hormone in sex difference in morphine modulation of CHS (Whitacre *et al*, 1999; McMurray, 2001).

A final key feature of these investigations is that the increased sensitivity to morphine observed in females generalized to other μ-opioid drugs with varying pharmacologic properties. For instance, whereas morphine and etorphine are both highly selective μ-opioid agonists (Aceto *et al*, 1997; Emmerson *et al*, 1994), buprenorphine also displays activity as an antagonist at κ and δ receptors (Negus and Dykstra, 1988; Tzschentke, 2002). Furthermore, buprenorphine is classified as a low-efficacy agonist at the μ-opioid receptor (Tzschentke, 2002), because it antagonizes the action of higher-efficacy opioid agonists such as morphine or etorphine. Morphine, etorphine, and buprenorphine also have different half-lives in plasma, with an order of buprenorphine > morphine > etorphine (Aceto *et al*, 1997; Gades *et al*, 2000). These differences in selectivity, efficacy, and pharmacokinetics demonstrate that sex differences in opioid modulation of the CHS response are generalizable across a range of μ-opioids and are not specific to the unique pharmacologic properties of one μ-opioid drug. Nevertheless, such pharmacologic differences may account for the discrepancy in time course and magnitude of CHS enhancement following treatment with morphine, etorphine, and buprenorphine (see **Figs 1, 2**).

The present findings represent a significant contribution to the literature concerning sex differences in cutaneous immune responses and pharmacologic modulation of the CHS response. Our studies demonstrate that although there are no significant sex differences in the CHS response following vehicle treatment, there are profound differences in sensitivity to opioids with females demonstrating significantly potentiated inflammation compared to males across a broad range of doses of morphine, buprenorphine, and etorphine. These effects appear specific to DNFB-induced CHS because morphine has no effect on croton oil-induced inflammation. Furthermore, morphine enhancement of CHS depends on the activation of brain μ-opioid receptors and on gonadal hormone milieu. Similar sex differences have been reported in both morphine's physiologic effects (Sarton *et al*, 1999; Sarton *et al*, 2000; Zun *et al*, 2002) and the magnitude of the CHS response (Rees *et al*, 1989) in humans. Given the apparently similar dynamic in morphine sensitivity between humans and our rat model, these data suggest the likelihood that morphine may exacerbate cutaneous allergic responses to a greater extent in women than men. Because CHS is widely studied as a prototype delayed-type hypersensitivity response, it is possible that opioids may produce sexually dimorphic modulation of a range of delayed-type hypersensitivity-dependent immune responses, including host defense and autoimmune susceptibility. Overall, these data suggest the need to consider sex as a determinant of opioid-induced immunomodulation.

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