Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 75 (2003) 235–245
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Agonist/antagonist properties of nalbuphine, butorphanol and (−)-pentazocine in male vs. female rats

R.M. Craft*, D.M. McNiel

Department of Psychology, Washington State University, CB 644820, Pullman, WA 99164-4820, USA

Received 29 November 2002; received in revised form 10 March 2003; accepted 26 March 2003

Abstract

To determine whether sex differences in the effects of mixed-action opioids could be due to differential activity at mu or kappa receptors, agonist/antagonist properties of nalbuphine, butorphanol and (−)-pentazocine were compared in male vs. female rats using a diuresis test. In water-loaded rats (2-h test), nalbuphine and (−)-pentazocine dose-dependently increased urination similarly in both sexes, whereas butorphanol increased urination more in females than in males on a ml/kg basis. The diuretic effects of all three opioids were at least partially blocked by the kappa receptor-selective antagonist nor-binaltorphimine (nor-BNI, 5 mg/kg) in both sexes. Kappa receptor-mediated antagonism of diuresis induced by U69,593 (0.56 mg/kg) was only observed with butorphanol in males. In water-loaded rats (1-h test), nalbuphine did not suppress, and butorphanol and (−)-pentazocine significantly suppressed urination in males only; all three mixed-action opioids dose-dependently blocked the antidiuretic effect of the selective mu agonist fentanyl (0.056 mg/kg) in both sexes. The ability of nalbuphine and (−)-pentazocine to block fentanyl-induced antidiuresis was not affected by pretreatment with nor-BNI in either sex. In contrast, the ability of butorphanol to block fentanyl-induced antidiuresis was attenuated by pretreatment with nor-BNI in males but not in females. These results suggest that sex differences in the effects of these mixed-action opioids are primarily due to their greater relative efficacy at the mu receptor in male than in female rats; butorphanol also may have greater efficacy at kappa receptors in females than in males.

Keywords: Diuresis; Urination; Sex differences; Gender; Opioids

1. Introduction

Opioid agonists such as nalbuphine, butorphanol and pentazocine produce differential analgesia in male vs. female humans (Gear et al., 1996a,b, 1999, 2000), monkeys (Negus and Mello, 1999) and rats (Cook et al., 2000; Craft and Bernal, 2001). Specifically, these opioids produced greater analgesia in women than in men undergoing dental surgery, but were more potent and in some cases more efficacious in male animals than in females using tests of acute thermal nociception. Because these agonists bind to multiple opioid receptor types, sex differences in their behavioral effects could be due to sex differences in their relative affinity for and/or efficacy at one or more receptor types.

Although not well-characterized in females of any species, nalbuphine has been shown to be a relatively low efficacy mu agonist in male rats, monkeys and humans. That is, nalbuphine produces lesser maximal effects than opioids such as morphine and fentanyl, and can block the effects of these higher efficacy mu agonists (Walker et al., 1993; Gerak et al., 1994; Morgan et al., 1999; Jones et al., 1999). Nalbuphine also may act as an antagonist of high-efficacy kappa agonists such as U50,488, spiradoline and enadoline in male animals (Dykstra, 1990; Gerak et al., 1994; Carey and Bergman, 2001; Smith and French, 2002). Butorphanol may act as an agonist or antagonist at mu and kappa opioid receptors in male rats, monkeys and humans, depending on the procedure (Leander, 1983c; Dykstra, 1990; Zacny et al., 1994; Greenwald and Stitzer, 1998; Wongchanapai et al., 1998; Vivian et al., 1999; but see Butelman et al., 1995), suggesting that it has relatively higher efficacy than nalbuphine at mu and kappa receptors. Similarly, pentazocine has variously been shown to act as a kappa agonist/antagonist in male mice (Suzuki et al., 1991; Chien and Pasternak, 1995), and a mu agonist/antagonist in male rats, monkeys and humans (Jasinski et al., 1970; Picker et al., 1992; Zacny et al., 1998; but see Dykstra, 1990).

* Corresponding author. Tel.: +1-509-335-5040; fax: +1-509-335-5043.
E-mail address: craft@wsu.edu (R.M. Craft).

0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00076-5

The purpose of the present study was to determine whether there are sex differences in the kappa vs. mu receptor-mediated effects of these opioids in rats. The dependent measure chosen was urination. Kappa agonists have been shown to increase urination (Leander, 1983a,b; Shearman and Tolcsvai, 1986; Leander et al., 1987), whereas mu agonists have been shown to suppress urination (Huidobro, 1978; Takemori et al., 1988; Soulard et al., 1996). Thus, whereas the antinociceptive effects of these mixed-action agonists have been found to be primarily mu receptor-mediated in common rodent and primate models of pain (e.g., Zimmerman et al., 1987; Walker et al., 1993; Butelman et al., 1995), their activity at both mu and kappa receptors may be characterized using a diuresis assay. Each of the three mixed-action opioids was examined for its diuretic (kappa agonist) effects in normally hydrated and water-loaded male and female rats using a 2-h test (Leander, 1983a,b; Leander et al., 1987; Soulard et al., 1996). Each mixed-action opioid was also tested for antidiuretic (mu agonist) effects in water-loaded rats using a 1-h test (Huidobro, 1978; Leander, 1983b; Soulard et al., 1996). Apparent kappa agonist effects were confirmed by determining whether they could be blocked by pretreatment with the kappa receptor-selective antagonist nor-binaltorphimine (nor-BNI). To further compare the kappa vs. mu receptor-mediated efficacy of each mixed-action opioid in males vs. females, each opioid was examined for its ability to antagonize the diuretic effect of the selective, high-efficacy kappa agonist U69,593 (Lahti et al., 1985), and for its ability to antagonize the antidiuretic effect of the selective, high-efficacy mu agonist fentanyl. When antagonism was observed, we attempted to reverse it with β-FNA or nor-BNI, to determine if the antagonism was functional—that is, attributable to the fact that mu and kappa receptor activation leads to decreases and increases in urination, respectively—effects that oppose each other when mu and kappa agonists are combined. For example, antagonism of U69,593's diuretic effects by any of the three mixed-action opioids that could be reversed by blocking mu receptors with β-FNA would indicate that the antagonism was functional in nature—presumably due to the mu receptor-mediated, antidiuretic effect of the mixed-action opioid, rather than due to its activity at kappa receptors.

We have shown previously that when sex differences in body weight are taken into account, relatively kappa receptor-selective agonists such as U69,593, U50,488 and (−)-bremazocine produced similar diuretic effects in normally hydrated male and female rats (Craft et al., 2000). In that study, butorphanol and (−)-pentazocine produced more modest diuresis in both sexes, suggesting that they have lower efficacy at the kappa receptor compared with drugs such as U69,593, or that they have mu agonist (i.e., antidiuretic) effects that limit their diuretic effects. Nalbuphine's effects on urination have not been compared in males vs. females.

2. Methods

2.1. Subjects

Adult Sprague–Dawley rats (bred in-house from Taconic stock, Germantown, NY), approximately 3–6 months old, that had been previously tested for antinociception with vehicle or an opioid or cannabinoid agonist (>1 week prior to the present study), served as subjects. Rats were housed in same-sex pairs, males and females in separate, adjacent rooms. Food and water were available ad libitum except during testing. Animal quarters were maintained at 21.5 ± 2.0 °C, on a 12:12 h light/dark cycle, with lights on at 0600 h. Rats were tested between approximately 0800 and 1300 h.

2.2. Apparatus

Urine was collected in stainless steel pans (each covered with a wire screen to prevent feces from collecting in the pan) in standard rodent operant chambers. During testing, chamber fans were activated, but no lights or levers were active.

2.3. Drugs

Fentanyl HCl, (−)-pentazocine succinate, β-FNA, nor-BNI, naltrexone HCl and U69,593 were obtained from the National Institute on Drug Abuse (Rockville, MD). Butorphanol tartrate was purchased from Sigma (St. Louis, MO). Nalbuphine HCl was purchased from RBI (Natick, MA) and ICN (Aurora, OH). Fentanyl, β-FNA, nor-BNI, naltrexone, butorphanol and nalbuphine were dissolved in physiological saline. (−)-Pentazocine was dissolved in distilled water. U69,593 was dissolved in EtOH to which distilled water was added, for a final EtOH concentration of 9.5%. A 9.5% ethanol solution served as the vehicle for U69,593; saline or distilled water served as the vehicle for all other drugs. All drugs were administered in volumes of 1 ml/kg, except in cases of solubility limitations (nalbuphine and β-FNA could only be made in 8–10 mg/ml maximal concentrations). All drugs were administered subcutaneously. All agonists and naltrexone were administered immediately before testing. β-FNA and nor-BNI were administered 24 h pretest, as they have previously been shown to be maximally or near-maximally effective and receptor-selective at this interval (Zimmerman et al., 1987; Butelman et al., 1993; Craft et al., 2001).

2.4. Procedure

All procedures used in this study were approved by the IACUC at Washington State University, and meet the guidelines set forth in the NIH Guide for Care and Use of Laboratory Animals (Publication #85-23, revised 1985). When testing kappa agonist/antagonist effects, rats were injected with one or more drugs and in some cases water-

loaded by administering warm tap water orally (20 ml/kg body weight). Rats were then immediately placed into individual chambers; urine was measured to the nearest 0.05 ml 2 h later. When testing mu agonist/antagonist effects, rats were water-loaded (20 ml/kg body weight), injected with one or more test compounds and then placed into individual chambers; urine was measured to the nearest 0.05 ml 1 h later. It has been shown previously that robust diuretic effects of kappa agonists can be observed using a 2- to 5-h test in either normally hydrated or water-loaded rats (e.g., Leander, 1983a), but that mu agonists are most effective when examined in a 1-h test in water-loaded rats, as their antidiuretic effects are relatively short-lived (Huidobro, 1978; Takemori et al., 1988). In addition, Leander et al. (1987) previously showed that diuretic effects of relatively low efficacy kappa agonists depended on the hydration condition used, so we tested both normally hydrated and water-loaded conditions. When testing agonists alone, the same rats were tested at each dose or dose combination of a given agonist; tests were conducted no more than twice/week (e.g., Mon, Thur or Tue, Fri). When testing the irreversible antagonists alone or in combination with agonists, separate rats were used for each treatment group and each rat was tested only once.

2.5. Data analysis

To adjust for significant sex differences in body size, all urine values in milliliters were transformed to milliliters per kilogram body weight (Leander, 1987; Craft et al., 2000). Milliliters per kilogram data for each mixed-action opioid were analyzed via ANOVA [e.g., 2 (sex) × 5–6, repeated (dose) × 2 (hydration condition)]. Kappa antagonist effects of each mixed-action opioid were analyzed via ANOVA [2 (sex) × 5–6, repeated (dose)]. Mu agonist/antagonist effects of each mixed-action opioid were analyzed via ANOVA [2 (sex) × 5–6, repeated (dose) × 2 (fentanyl dose)]. Student–Newman–Keuls (SNK) post hoc tests were used when significant sex differences (or sex interactions) were obtained in the ANOVA, to determine at what doses sex differences were significant. Dunnett's test was used to determine what doses of a given opioid, relative to vehicle, significantly altered urination in each sex. β-FNA and nor-BNI data were analyzed via ANOVA (Sex × Antagonist × Agonist), followed by Dunnett's test, as each dose combination was compared only to control. Significance level for all tests was $P \leq .05$.

3. Results

3.1. Diuretic effects of mixed-action opioids in males vs. females

Fig. 1 (points above "saline") shows that, in the absence of drug, water-loaded rats urinated significantly more than normally hydrated rats, but there were no sex differences in these baselines. Rats injected subcutaneously with a slightly larger volume of saline (as a control for the increased volume necessary to administer the 30 mg/kg nalbuphine dose; not shown) did not urinate significantly more than those shown in Fig. 1, nor did those administered the 9.5% ethanol vehicle used to dissolve U69,593 (see Fig. 4). Nalbuphine (top panel) dose-dependently increased urination in both normally hydrated and water-loaded rats [Dose: $F(4,120) = 40.60$, $P < .001$], with no significant sex differences. The diuretic effects of nalbuphine were slightly greater under the normally hydrated condition than under the water-loaded condition, as indicated by the relatively steeper slope of the dose–effect curve in normally hydrated compared to water-loaded rats, particularly in males.

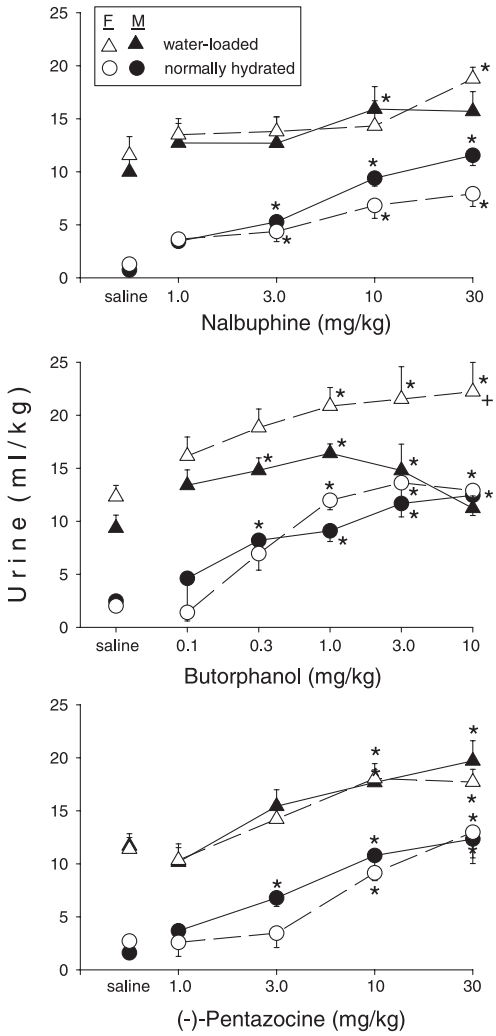

Fig. 1. Diuretic effects of three mixed-action opioids in female (F) vs. male (M) rats. Rats were either normally hydrated or water-loaded; urine was collected for 2 h following subcutaneous saline or opioid injection. Each point is the mean ± 1 S.E.M. of 7–10 rats. * Significantly different from same-sex, same-hydration controls, $P \leq .05$, SNK; † significantly different from males within same hydration condition, $P \leq .05$, SNK.

Butorphanol (middle panel) also dose-dependently increased urination in male and female rats. Similar to nalbuphine, butorphanol's effect was greater in normally hydrated than in water-loaded rats, a statistically significant difference in this case [Dose × Hydration condition: $F(5,170) = 3.84$, $P = .003$]. Butorphanol-induced diuresis was blunted in water-loaded males relative to normally hydrated males. Thus, under the water-loaded condition only, butorphanol-induced urination was significantly greater in females than in males [Sex × Hydration condition: $F(1,34) = 7.23$, $P = .011$].

(−)-Pentazocine (bottom panel) also dose-dependently increased urination [Dose: $F(4,96) = 32.57$, $P < .001$], but produced similar increases under both normally hydrated and water-loaded conditions, with no sex differences.

3.2. Receptor-selective antagonism of diuresis

Doses of nor-BNI and β-FNA were chosen based on their ability to selectively antagonize agonists that are known to act at either kappa or mu receptors, respectively. Fig. 2 shows that 5 mg/kg nor-BNI blocked the diuretic effects of the selective kappa agonist U69,593 (top panel), but did not block the antidiuretic effects of the selective mu agonist fentanyl (bottom panel), in rats of both sexes. Conversely, 20 mg/kg β-FNA completely blocked the antidiuretic effects of the selective mu agonist fentanyl (bottom panel), but did not block the diuretic effects of the selective kappa agonist U69,593 (top panel), in rats of both sexes [Antagonist × U69,593: $F(2,94) = 9.85$, $P < .001$; Antagonist × Fentanyl: $F(2,83) = 21.14$, $P < .001$].

Fig. 3 (top panel) shows that nor-BNI blocked the diuretic effects of 30 mg/kg nalbuphine in rats of both sexes, whereas β-FNA did not [Antagonist × Nalbuphine: $F(2,54) = 6.52$, $P = .003$]. Similarly, the diuretic effects of 10 mg/kg butorphanol were significantly attenuated by nor-BNI but not by β-FNA in rats of both sexes (Fig. 3, middle panel) [Antagonist × Butorphanol: $F(2,69) = 4.92$, $P = .01$]. The diuretic effects of 30 mg/kg (−)-pentazocine also were attenuated by nor-BNI (Fig. 3, bottom panel) [Antagonist × Pentazocine: $F(2,67) = 4.76$, $P = .012$], but only significantly so in females. In males, β-FNA and nor-BNI had essentially the same effect on (−)-pentazocine-induced diuresis, both decreasing urination nonsignificantly compared to (−)-pentazocine-treated controls. A higher dose of nor-BNI, 10 mg/kg, did not further antagonize (−)-pentazocine's diuretic effect in rats of either sex (not shown). However, the nonselective opioid antagonist naltrexone (1–30 mg/kg) dose-dependently and more completely antagonized (−)-pentazocine's diuretic effect in both sexes (data not shown).

3.3. Kappa antagonist effects of mixed-action opioids

Fig. 4 shows that at the doses tested, nalbuphine (top panel, squares) did not antagonize the diuretic effects of the high efficacy, kappa selective agonist U69,593 in rats of either sex. Higher doses of nalbuphine were not tested due to

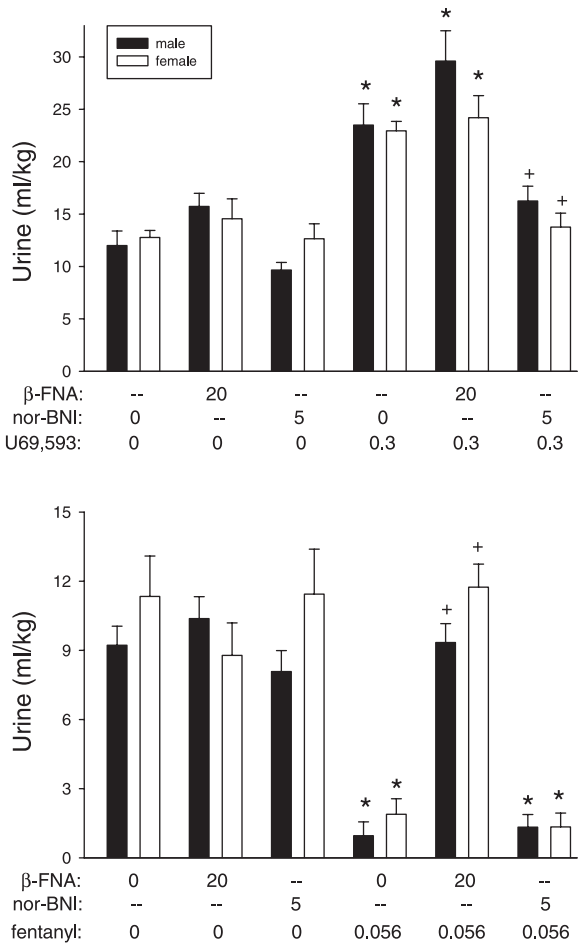

Fig. 2. Selectivity of the opioid antagonists β-FNA (mu) and nor-BNI (kappa) in male and female rats. Rats were water-loaded; antagonists were administered subcutaneously 24 h pretest and agonists were administered subcutaneously immediately pretest. Top panel: Antagonism of the selective kappa agonist U69,593 (2-h test). Bottom panel: Antagonism of the selective mu agonist fentanyl (1-h test). Doses shown on abscissae are in mg/kg; "0" = vehicle, "–" = no injection. Each bar is the mean ± 1 S.E.M. of 5–13 rats. * Significantly different from same-sex 0–0 controls; † Significant antagonism relative to same-sex agonist-alone (0–0.3, 0–0.056), $P \leq .05$, Dunnett's.

solubility limitations. In contrast, butorphanol (middle panel, squares) significantly antagonized U69,593's diuretic effect in males but not in females [Sex × Butorphanol dose: $F(5,90) = 5.02$, $P < .001$]. It should be noted, however, that in females the effect of butorphanol alone was comparable to the effect of U69,593 alone; thus, antagonism could not be observed in females. (−)-Pentazocine (bottom panel, squares) dose-dependently but only weakly antagonized U69,593's diuretic effect in both sexes [Pentazocine dose: $F(4,32) = 4.65$, $P = .005$ (males); $F(4,32) = 2.80$, $P = .05$ (females)], although individual points were not significantly different from control.

The ability of butorphanol and (−)-pentazocine to attenuate the diuretic effects of U69,593 could be due to (a) their competitive binding at the receptor at which U69,593 acts (pharmacological antagonism, presumably at the kappa

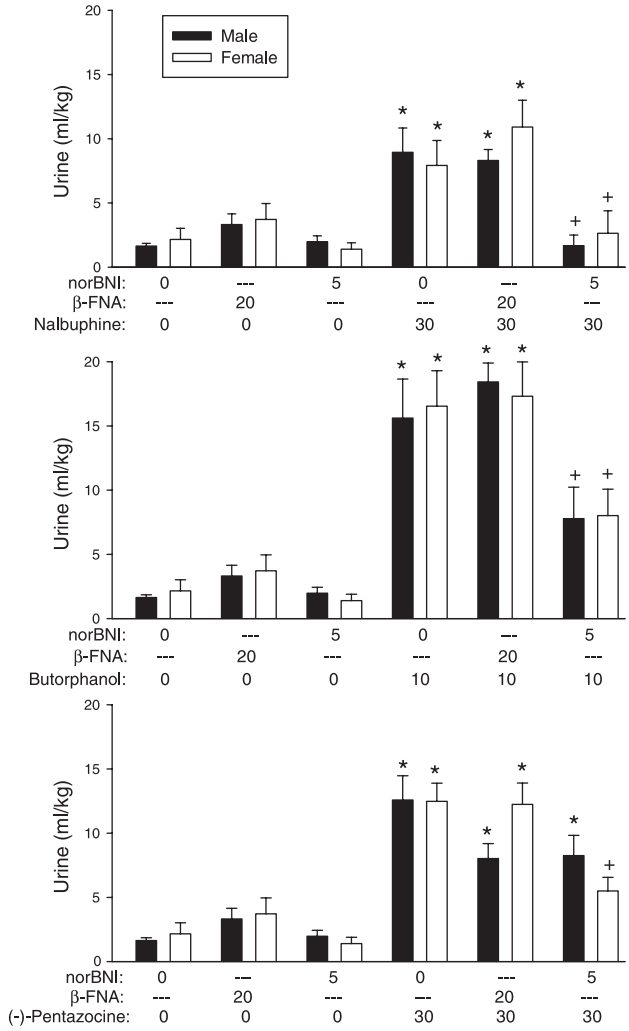

Fig. 3. Antagonism of the diuretic effects of nalbuphine (top panel), butorphanol (middle panel) and (−)-pentazocine (bottom panel) by the kappa and mu receptor-selective antagonists nor-BNI and β-FNA in male vs. female rats. Rats were normally hydrated; antagonists were administered subcutaneously 24 h pretest and agonists were administered subcutaneously immediately pretest, and urine was measured at 2 h. Doses shown on abscissae are in mg/kg; "0" = vehicle, " − " = no injection. Each bar is the mean ± 1 S.E.M. of five to nine rats; saline and antagonist-alone values in each panel are the same data. * Significantly different from same-sex 0 – 0 controls; † Significant antagonism relative to same-sex agonist-alone (0 – 30, 0 – 10, 0 – 30), $P \leq .05$, Dunnett's.

opioid receptor), or (b) their antidiuretic effects (functional antagonism, presumably mu receptor-mediated). To test the latter possibility, rats were pretreated with the mu receptor-selective antagonist β-FNA, to determine whether blocking any mu-mediated, antidiuretic effects of butorphanol and (−)-pentazocine would eliminate their antagonism of U69,593-induced diuresis. Fig. 5 (top panel) shows that in male rats—the only sex in which butorphanol antagonized U69,593—pretreatment with β-FNA did not eliminate butorphanol's antagonism of U69,593 [Butorphanol × U69,593: $F(1,62) = 63.64$, $P < .001$; no Butorphanol × U69,593 × β-FNA interaction]. Pretreatment with β-FNA did appear to attenuate (−)-pentazocine's antagonism of U69,593 in both sexes; however, because (−)-pentazocine's antagonism of U69,593 was only marginally significant, reversal of this effect was difficult to demonstrate. Fig. 5 (bottom) illustrates β-FNA's apparent but nonsignificant reversal of (−)-pentazocine's antagonism of U69,593 in female rats.

3.4. Mu agonist/antagonist effects of mixed-action opioids

To examine mu agonist/antagonist effects of nalbuphine, butorphanol and (−)-pentazocine, each opioid was examined alone and in combination with the high efficacy, mu-selective agonist fentanyl, using a 1-h test in water-loaded rats. Fig. 6 shows that 0.056 mg/kg fentanyl alone (triangles above "saline") almost completely abolished urination in

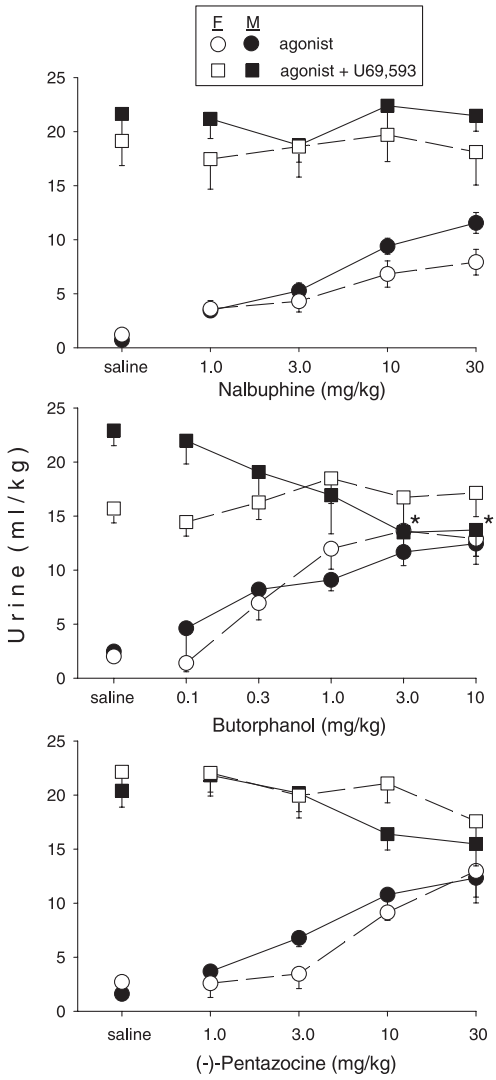

Fig. 4. Kappa antagonist effects of three mixed-action opioids in female (F) vs. male (M) rats. Rats were normally hydrated; each mixed-action agonist was given alone (replotted from Fig. 1) or in combination with the high-efficacy kappa receptor-selective agonist U69,593 (0.56 mg/kg sc); urine was measured at 2 h. Each point is the mean ± 1 S.E.M. of 5 – 10 rats. * Significantly different from same-sex 0 – 0 controls, $P \leq .05$, Dunnett's.

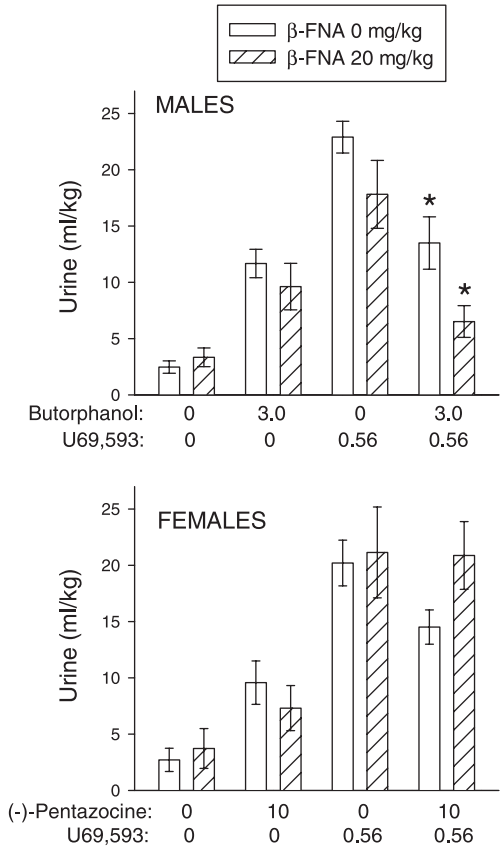

Fig. 5. Reversal of the kappa antagonist effects produced by butorphanol (top panel) and (−)-pentazocine (bottom panels) by the mu receptor-selective antagonist β-FNA. Rats were normally hydrated, β-FNA was administered 24 h pretest and the agonists were administered immediately pretest; urine was measured at 2 h. Doses shown on abscissae are in mg/kg. Each bar is the mean ± 1 S.E.M. of 5–11 rats. * Significant antagonism relative to U69,593 alone within the same β-FNA condition, $P \leq .05$, Dunnett's.

water-loaded rats (compare to circles above "saline"), with no significant sex differences. Nalbuphine given alone (top panel, circles) did not significantly decrease urination in females or males. When given in combination with fentanyl (triangles), nalbuphine dose-dependently blocked fentanyl's antidiuretic effect in both sexes [Nalbuphine dose × Fentanyl dose: $F(4,140) = 10.85$, $P < .001$]. Nalbuphine + fentanyl-treated females urinated more than their male counterparts [Sex: $F(1,17) = 5.21$, $P = .036$], with post hoc tests revealing a significant difference only at 30 mg/kg nalbuphine, which completely blocked fentanyl-induced antidiuresis in females only.

Fig. 6 (middle panel) shows that when given alone (circles), butorphanol decreased urination primarily in males; when combined with fentanyl (triangles), butorphanol dose-dependently blocked fentanyl's antidiuretic effect in rats of both sexes [Butorphanol dose × Fentanyl dose × Sex: $F(5,170) = 3.25$, $P = .008$]. Given alone, butorphanol's antidiuretic effect was maximal in males at 3–10 mg/kg, doses that did not significantly affect urination in females [Butorphanol Dose × Sex: $F(5,85) = 5.49$, $P < .001$]. The antidiuretic effect of 3.0 mg/kg butorphanol in males was completely blocked by pretreatment with the mu-selective antagonist β-FNA [$t(1,13) = -3.58$, $P = .003$; data not shown]. Given in combination with fentanyl, butorphanol's antagonist effect was significant at 3–10 mg/kg in both sexes [Butorphanol dose: $F(5,85) = 10.16$, $P < .001$].

Fig. 6 (bottom panel) shows the effects of (−)-pentazocine given alone and in combination with fentanyl in males vs. females. Similar to butorphanol, (−)-pentazocine given alone (circles) dose-dependently decreased urination only in males. When given in combination with fentanyl (triangles), (−)-pentazocine antagonized fentanyl's antidiuretic effect in both sexes [Pentazocine dose × Fentanyl dose × Sex: $F(4,128) = 3.65$, $P = .007$]. Given alone, (−)-pentazocine's

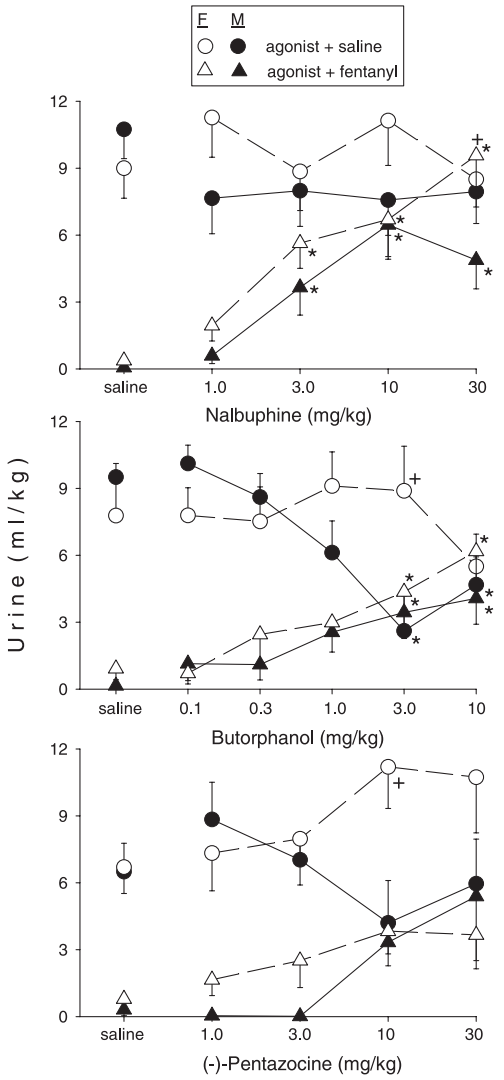

Fig. 6. Mu antagonist effects of three mixed-action opioids in female (F) vs. male (M) rats. Rats were water-loaded, each mixed-action opioid was given in combination with saline or the high-efficacy mu receptor-selective agonist fentanyl (0.056 mg/kg sc); urine was measured at 1 h. Each point is the mean ± 1 S.E.M. of 8–10 rats. * Significantly different from same-sex 0–0 controls, $P \leq .05$, Dunnett's; †Significantly different from males within same fentanyl condition, $P \leq .05$, SNK.

antidiuretic effect was maximal in males at 10–30 mg/kg, doses that tended to *increase* urination in females [Pentazocine dose × Sex: $F(4,64) = 3.47$, $P = .013$]. The antidiuretic effect of 10 mg/kg (−)-pentazocine in males was completely blocked by the mu-selective antagonist β-FNA [$t(1,13) = -3.16$, $P = .008$; data not shown]. When combined with fentanyl, (−)-pentazocine's antagonist effect was maximal at 10–30 mg/kg and similar in males and females [Pentazocine dose: $F(4,64) = 5.79$, $P < .001$].

The ability of nalbuphine, butorphanol and (−)-pentazocine to attenuate the antidiuretic effects of fentanyl could be due to (a) their competitive binding at the receptor at which fentanyl acts (pharmacological antagonism, presumably at the mu opioid receptor), or (b) their diuretic effects (functional antagonism, presumably kappa receptor-mediated). To test the latter possibility, rats were pretreated with the kappa receptor-selective antagonist nor-BNI, to determine whether blocking the kappa receptor-mediated diuretic effects of the mixed-action opioids would eliminate their ability to antagonize fentanyl's antidiuretic effect. Fig. 7 (top panels) shows that in both male and female rats, pretreatment with nor-BNI did not alter nalbuphine's antagonism of fentanyl [Males, Nalbuphine × Fentanyl: $F(1,58) = 24.38$, $P < .001$, no Nalbuphine × Fentanyl × nor-BNI interaction; Females, Nalbuphine × Fentanyl: $F(1,53) = 13.58$, $P < .001$, no three-way interaction]. In contrast, Fig. 7 (middle panels) shows that pretreatment with nor-BNI eliminated butorphanol's antagonism of fentanyl in males but not in females [Males, Butorphanol × Fentanyl × nor-BNI: $F(2,94) = 4.68$, $P = .012$; Females, Butorphanol × Fentanyl: $F(2,99) = 5.32$, $P = .006$, no three-way interaction]. Nor-BNI pretreatment also revealed a mu agonist-like (antidiuretic) effect of 3.0 mg/kg butorphanol in female rats, although this effect was not statistically significant in the overall analysis.

Similar to nalbuphine, (−)-pentazocine's antagonism of fentanyl was not significantly altered by pretreatment with the kappa antagonist nor-BNI in males or in females (Fig. 7, bottom panels) [Males, Pentazocine × Fentanyl: $F(1,52) = 11.10$, $P = .002$; Females, Pentazocine × Fentanyl: $F(1,54) = 4.10$, $P = .048$; no three-way interactions].

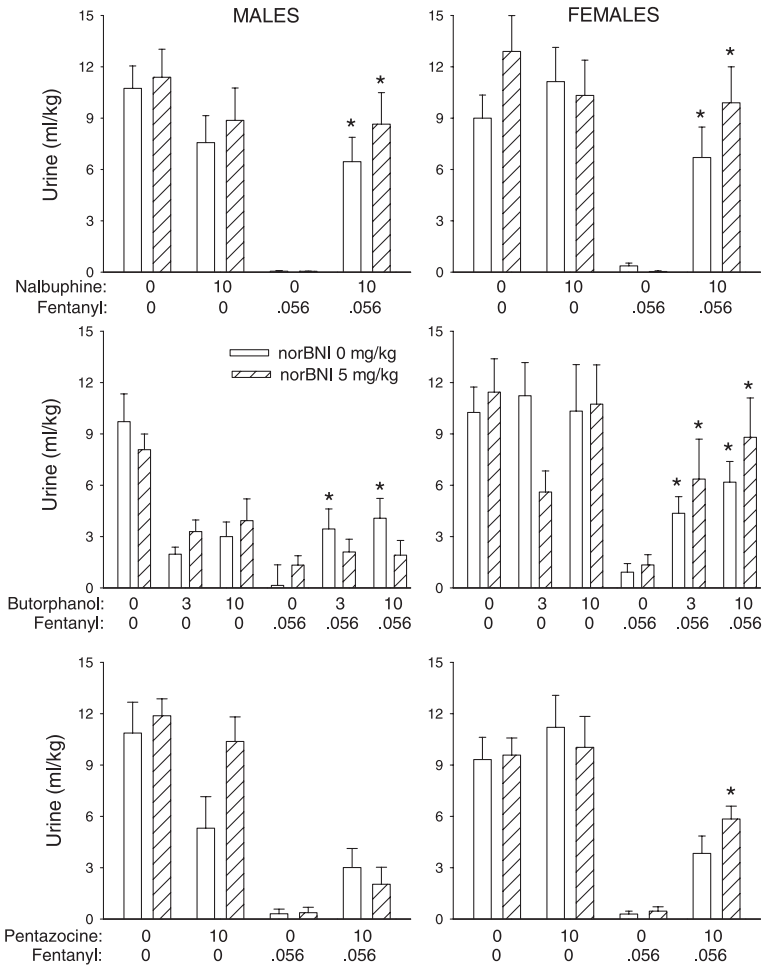

Fig. 7. Reversal of the mu antagonist effects produced by nalbuphine (top panels), butorphanol (middle panels) and (−)-pentazocine (bottom panels) by the kappa receptor-selective antagonist nor-BNI in male vs. female rats. Nor-BNI was administered 24 h pretest, and the agonists were administered to water-loaded rats immediately pretest; urine was measured at 1 h. Each bar is the mean ± 1 S.E.M. of 4–10 rats. * Significant antagonism relative to fentanyl alone (0–0.056) controls within the same nor-BNI condition, $P \leq .05$, Dunnett's.

4. Discussion

Table 1 summarizes the evidence for mu and kappa receptor-mediated effects of the three mixed-action opioids in male vs. female rats in the present study. All three mixed-action opioids produced kappa receptor-mediated agonist effects in both sexes, and two of three opioids (butorphanol and (−)-pentazocine) appeared to produce mu receptor-mediated agonist effects only in males. Only butorphanol produced clear kappa receptor-mediated antagonist effects, and this was observed only in males. In contrast, all three mixed-action opioids produced mu receptor-mediated antagonist effects: nalbuphine and (−)-pentazocine in both sexes, butorphanol only in females. These data suggest that (1) nalbuphine has intermediate efficacy at kappa receptors and low efficacy at mu receptors in both male and female rats, although its efficacy at mu receptors is somewhat greater in males than in females; (2) butorphanol has higher efficacy at kappa receptors in females than in males, and perhaps higher efficacy at mu receptors in males than in females; and (3) (−)-pentazocine has intermediate to low efficacy at kappa receptors in both sexes, and higher efficacy at mu receptors in males than in females. The present study therefore demonstrates that sex differences in the effects of some mixed-action opioids may be due to differential activity at mu or kappa receptors.

The characterization of nalbuphine as a relatively low efficacy mu agonist in rats of both sexes corroborates previous studies showing that nalbuphine is a relatively low efficacy mu agonist in pigeons, rats, monkeys and humans tested in a variety of procedures (Picker and Dykstra, 1989; Walker et al., 1993; Gerak et al., 1994; Zacny et al., 1997; Morgan et al., 1999; Jones et al., 1999). The present results also agree with previous studies showing that nalbuphine has kappa receptor-mediated effects in pigeons, rats and monkeys, although its efficacy is relatively low such that it often acts as an antagonist rather than as an agonist at kappa receptors (Dykstra, 1990; Leander, 1983b; Gerak et al., 1994; Holtzman and Steinfels, 1994; Smith and Picker, 1995; Brandt and France, 1996). In humans, there is little direct evidence available for examining kappa receptor mediation of nalbuphine's effects; however, a recent study indicates that nalbuphine also has relatively low efficacy at the human kappa opioid receptor (Remmers et al., 1999). The only significant sex differences in nalbuphine's effects in the present study were in the degree to which it antagonized fentanyl: nalbuphine antagonized fentanyl more completely in females than in males, suggesting that it has lower efficacy at the mu receptor in females than in males. This sex difference has been suggested previously in studies in which nalbuphine was a less efficacious antinociceptive agent in female than in male rats and monkeys (Negus and Mello, 1999; Cook et al., 2000; Craft and Bernal, 2001).

The present study demonstrates that butorphanol also produced effects via mu and kappa receptor activation, with some sex differences. Butorphanol produced nor-BNI-reversible diuresis in rats of both sexes. However, butorphanol-induced diuresis was significantly greater in females than in males under the water-loaded condition (2-h test), suggesting that butorphanol has greater efficacy at kappa receptors in females, and/or greater efficacy at mu receptors in males. In the 1-h test optimal for assaying mu agonist effects (Huidobro, 1978; Leander, 1983b; Soulard et al., 1996), butorphanol suppressed urination (in a β-FNA-reversible manner) in males but not in females. It should be noted that butorphanol probably possesses some mu agonist activity in females: when kappa receptors were blocked by nor-BNI, 3.0 mg/kg butorphanol did suppress urination somewhat in females (Fig. 7). This result suggests that butorphanol's kappa agonist (diuretic) effects may mask its mu agonist (antidiuretic) effects in females, even in the 1-h test. However, whereas butorphanol antagonized the antidiuretic effect of fentanyl in both sexes, this antagonism was reversed by nor-BNI in males, suggesting that it was mu receptor-mediated only in females. Taken together, these results indicate that, similar to nalbuphine, butorphanol has greater efficacy at the mu receptor in males than in females. Butorphanol also antagonized the diuretic effects of U69,593

Table 1
Summary of evidence for mu and kappa agonist/antagonist effects of three mixed-action opioids in male (M) and female (F) rats

	Nalbuphine	Butorphanol	(−)-Pentazocine
Kappa partial agonist?	Yes (M and F): produced nor-BNI-reversible diuresis	Yes (M and F): produced nor-BNI-reversible diuresis	Yes (M and F): produced nor-BNI-reversible diuresis[a]
Kappa antagonist?	No (M and F): no block of U69,593 diuresis	Yes (M only): blocked U69,593 diuresis; not β-FNA-reversible	No (M and F): weakly blocked U69,593 diuresis[b]
Mu partial agonist?	No (M and F): no antidiuresis	Yes (M only): produced β-FNA-reversible antidiuresis	Yes (M only): produced β-FNA-reversible antidiuresis
Mu antagonist?	Yes (M and F): blocked fentanyl antidiuresis[c]; not nor-BNI-reversible	Yes (F only): blocked fentanyl antidiuresis; not nor-BNI-reversible	Yes (M and F): blocked fentanyl antidiuresis; not nor-BNI-reversible

[a] (−)-Pentazocine-induced diuresis also appeared to be mediated in part by mechanisms other than kappa receptor activation: its diuretic effect was only significantly antagonized by nor-BNI in females, although naltrexone was effective in both sexes.

[b] β-FNA appeared to reverse (−)-pentazocine's antagonism of U69,593, but this effect was not statistically significant (see Fig. 5).

[c] The highest dose of nalbuphine antagonized fentanyl to a significantly greater extent in females than in males.

in males but not in females. This antagonism was not reversed by β-FNA, suggesting that butorphanol has lower efficacy at kappa receptors in males than in females. One limitation is that in females, butorphanol produced as much urination as U69,593 did, making it difficult to observe antagonism of U69,593. These results extend previous studies showing that butorphanol may act as an agonist or antagonist at both mu and kappa receptors in (primarily male) rats, monkeys and humans (Dykstra, 1990; Zacny et al., 1994; Greenwald and Stitzer, 1998; Wongchanapai et al., 1998; Vivian et al., 1999; but see Butelman et al., 1995). Previous studies comparing butorphanol's analgesic effects in male and female animals also showed that it had greater efficacy in males (Negus and Mello, 1999; Cook et al., 2000; Craft and Bernal, 2001), but activity at mu vs. kappa receptors was not compared. The present study suggests that butorphanol has relatively greater efficacy at mu receptors *and* lesser efficacy at kappa receptors in males than in females.

The third mixed-action opioid examined, (−)-pentazocine, also produced some sex-dependent effects on the diuresis assay. (−)-Pentazocine dose-dependently increased urination in both sexes, to a similar extent. Nor-BNI was at least partially effective in blocking this diuresis, suggesting that (−)-pentazocine has some efficacy at kappa receptors. (−)-Pentazocine also weakly blocked the diuretic effects of U69,593. However, this antagonist effect appeared to be reversed by blocking mu receptors with β-FNA, suggesting that (−)-pentazocine was not a "pharmacological" kappa antagonist. When tested for mu agonist effects (1-h test), (−)-pentazocine decreased urination (in a β-FNA-reversible manner) only in males, the same sex difference observed with butorphanol. (−)-Pentazocine also blocked fentanyl's antidiuretic effect (and this antagonism was not reversed by nor-BNI) in both sexes, suggesting that (−)-pentazocine has relatively low efficacy at mu receptors. Taken together, these results suggest that (−)-pentazocine has relatively low efficacy at mu receptors in both sexes, but greater efficacy in males than in females. These results corroborate previous studies showing that pentazocine acts as a mu and/or kappa agonist/antagonist in (primarily male) mice, rats, monkeys and humans (Suzuki et al., 1991; Chien and Pasternak, 1995; Jasinski et al., 1970; Picker et al., 1992; Zacny et al., 1998; but see Dykstra, 1990). One previous study also demonstrated that (−)-pentazocine had greater analgesic efficacy in male than in female rats (Craft and Bernal, 2001), but activity at mu vs. kappa receptors was not examined. The present study suggests that (−)-pentazocine has greater efficacy at mu receptors in males, but similar efficacy at kappa receptors in males and females. It should also be noted that there may be sex differences in (−)-pentazocine's effects at receptors other than mu and kappa (or other than opioid), since nor-BNI did not completely antagonize diuresis in males. The fact that naltrexone antagonized (−)-pentazocine-induced diuresis in both sexes suggests that there may be a delta receptor-mediated component to this drug's diuretic effect. Sigma agonist effects of pentazocine also have been reported (e.g., Chien and Pasternak, 1995); sex differences in sigma agonist effects have not been examined.

Nalbuphine, butorphanol and pentazocine are commonly used to treat moderate clinical pain. Contrary to many studies in animals, one laboratory has demonstrated that these opioids are more potent and in some cases more effective in women than in men, against postdental-surgery pain (Gear et al., 1996a,b, 1999, 2000). It has been postulated that these mixed-action opioids produce greater analgesia in women because they produce greater kappa receptor-mediated activity in women than in men (e.g., Miaskowski and Levine, 1999; Gear et al., 2000). The present results indicate that butorphanol indeed may have greater efficacy at kappa receptors in females than in males. However, the other two drugs did not have differential kappa receptor-mediated activity in males and females; therefore this feature alone is unlikely to explain sex differences in the effects of all three drugs. In contrast, all three drugs appeared to have greater efficacy at mu receptors in males than in females. It is not known to what extent the mu vs. kappa receptor-mediated effects of these drugs contributes to analgesia in humans. In animals, antagonism studies indicate that their antinociceptive effects are predominantly mu receptor-mediated—although it should be noted that most of these studies have been conducted in male animals only. In humans, studies of the subjective, physiological and psychomotor effects of these mixed-action opioids demonstrate that at analgesic doses, nalbuphine is very similar to morphine, a more selective mu agonist (Zacny et al., 1997), whereas butorphanol and pentazocine produce both morphine-like effects and other effects that may be kappa-like (e.g., dysphoria, psychomotor impairment) (Zacny et al., 1994; 1998; Walker et al., 2001). Future studies using antagonist approaches will be needed to confirm whether the mechanisms of action of these opioids in humans are as similar to those in animals as they appear to be from the existing data.

The present study, which is the first to explicitly compare both mu and kappa receptor-mediated activity of mixed-action opioids in males vs. females, suggests that there are sex differences in (1) the relative efficacy of all three mixed-action opioids at mu receptors, with greater efficacy in males than in females, and (2) the relative efficacy of butorphanol at kappa receptors, with greater efficacy in females than in males. One limitation that should be noted for cases in which the mixed-action opioids did not significantly antagonize higher efficacy agonists is that the doses of each mixed-action agonist examined may not have been high enough to occupy sufficient numbers of receptors. Also, this assay may not have been sensitive enough to detect low-efficacy mu agonist effects (e.g., of nalbuphine). Finally, it is also possible that the apparent sex differences in opioid efficacy observed in this study actually reflect sex differences in the affinity of the mixed-action opioids for the various opioid receptors, or the opioids' mu vs. kappa receptor selectivity.

Acknowledgements

The authors thank A. Trockel, J. Clark and D. Reid for technical assistance. This research was supported by NIDA DA10284, and by funds provided for medical and biological research from the State of Washington Initiative Measure No. 171.

References

Brandt MR, France CP. Discriminative stimulus effects of enadoline in pigeons. J Pharmacol Exp Ther 1996;277:960–7.

Butelman E, Negus SS, Ai Y, de Costa BR, Woods JH. Kappa opioid antagonist effects of systemically administered nor-binaltorphimine in a thermal antinociception assay in rhesus monkeys. J Pharmacol Exp Ther 1993;267:1269–76.

Butelman E, Winger G, Zernig G, Woods J. Butorphanol: characterization of agonist and antagonist effects in rhesus monkeys. J Pharmacol Exp Ther 1995;272:845–53.

Carey GJ, Bergman J. Enadoline discrimination in squirrel monkeys: effects of opioid agonists and antagonists. J Pharmacol Exp Ther 2001;297: 215–23.

Chien C-C, Pasternak G. (−)-Pentazocine analgesia in mice: interactions with a σ receptor system. Eur J Pharmacol 1995;294:303–8.

Cook C, Barrett A, Roach E, Bowman J, Picker M. Sex-related differences in the antinociceptive effects of opioids: importance of rat genotype, nociceptive stimulus intensity, and efficacy at the mu opioid receptor. Psychopharmacology 2000;150:430–42.

Craft RM, Bernal SA. Sex differences in opioid antinociception: kappa and 'mixed action' agonists. Drug Alcohol Depend 2001;63:215–28.

Craft RM, Ulibarri CM, Raub DJ. Kappa opioid-induced diuresis in female vs. male rats. Pharmacol Biochem Behav 2000;65:53–9.

Craft RM, Tseng AH, McNiel DM, Furness MS, Rice KC. Receptor-selective antagonism of opioid antinociception in female vs. male rats. Behav Pharmacol 2001;12:591–602.

Dykstra L. Butorphanol, levallorphan, nalbuphine and nalorphine as antagonists in the squirrel monkey. J Pharmacol Exp Ther 1990;254:245–52.

Gear RW, Gordon NC, Heller PH, Paul S, Miaskowski C, Levine JD. Gender difference in analgesic response to the kappa-opioid pentazocine. Neurosci Lett 1996a;205:207–9.

Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD. Kappa-opioids produce significantly greater analgesia in women than in men. Nat Med 1996b;2:1248–50.

Gear R, Miaskowski C, Gordon N, Paul S, Heller P, Levine J. The kappa opioid nalbuphine produces gender- and dose-dependent analgesia and antianalgesia in patients with postoperative pain. Pain 1999;83:339–45.

Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD. Action of naloxone on gender-dependent analgesic and antianalgesic effects of nalbuphine in humans. J Pain 2000;1:122–7.

Gerak L, Butelman E, Woods J, France C. Antinociceptive and respiratory effects of nalbuphine in rhesus monkeys. J Pharmacol Exp Ther 1994; 271:993–9.

Greenwald MK, Stitzer ML. Butorphanol agonist effects and acute physical dependence in opioid abusers: comparison with morphine. Drug Alcohol Depend 1998;53:17–30.

Holtzman SG, Steinfels GF. Antagonism of the discriminative stimulus effects of the kappa-opioid agonist spiradoline. Psychopharmacology 1994;116:243–8.

Huidobro F. Antidiuretic effect of morphine in the rat: tolerance and physical dependence. Br J Pharmacol 1978;64:167–71.

Jasinski DR, Martin WR, Hoeldtke RD. Effects of short- and long-term administration of pentazocine in man. Clin Pharmacol Ther 1970;11: 385–403.

Jones H, Bigelow G, Preston K. Assessment of opioid partial agonist activity with a three-choice hydromorphone dose-discrimination procedure. J Pharmacol Exp Ther 1999;289:1350–61.

Lahti R, Mickelson M, McCall J, VonVoigtlander P. [^{3}H]U-69593 a highly selective ligand for the opioid κ receptor. Eur J Pharmacol 1985;109: 281–4.

Leander JD. A kappa opioid effect: increased urination in the rat. J Pharmacol Exp Ther 1983a;224:89–94.

Leander JD. Further study of kappa opioids on increased urination. J Pharmacol Exp Ther 1983b;227:35–41.

Leander JD. Evidence that nalorphine, butorphanol and oxilorphan are partial agonists at a κ-opioid receptor. Eur J Pharmacol 1983c;86:467–70.

Leander JD. Bremazocine-induced urination in rats of different sizes. Res Commun Chem Path Pharmacol 1987;57:141–4.

Leander JD, Hart JC, Zerbe RL. Kappa agonist-induced diuresis: evidence for stereoselectivity, strain differences, independence of hydration variables and a result of decreased plasma vasopressin levels. J Pharmacol Exp Ther 1987;242:33–9.

Miaskowski C, Levine JD. Does opioid analgesia show a gender preference for females? Pain Forum 1999;8:34–44.

Morgan D, Cook C, Smith M, Picker M. An examination of the interactions between the antinociceptive effects of morphine and various mu-opioids: the role of intrinsic efficacy and stimulus intensity. Anesth Analg 1999;88:407–13.

Negus S, Mello N. Opioid antinociception in ovariectomized monkeys: comparison with antinociception in males and effects of estradiol replacement. J Pharmacol Exp Ther 1999;290:1132–40.

Picker MJ, Dykstra LA. Discriminative stimulus effects of mu and kappa opioids in the pigeon: analysis of the effects of full and partial mu and kappa agonists. J Pharmacol Exp Ther 1989;249:557–66.

Picker MJ, Craft RM, Negus SS, Powell KR, Mattox SR, Jones SR, et al. Intermediate efficacy mu opioids: examination of their morphine-like stimulus effects and response rate-decreasing effects in morphine-tolerant rats. J Pharmacol Exp Ther 1992;263:668–81.

Remmers AE, Clark MJ, Mansour A, Akil H, Woods JH. Opioid efficacy in a C6 glioma cell line stably expressing the human kappa opioid receptor. J Pharmacol Exp Ther 1999;288:827–33.

Shearman GT, Tolcsvai L. Tifluadom-induced diuresis in rats, evidence for an opioid receptor-mediated central action. Neuropharmacology 1986; 25:853–6.

Smith MA, French AM. Age-related differences in sensitivity to the antinociceptive effects of kappa opioids in adult male rats. Psychopharmacology 2002;162:255–64.

Smith MA, Picker MJ. Examination of the kappa agonist and antagonist properties of opioids in the rat drug discrimination procedure: influence of training dose and intrinsic efficacy. Behav Pharmacol 1995;6:703–17.

Soulard CD, Guerif S, Payne A, Dahl SG. Differential effects of fedotozine compared to other κ agonists on diuresis in rats. J Pharmacol Exp Ther 1996;279:1379–85.

Suzuki T, Narita M, Misawa M, Nagase H. Pentazocine-induced biphasic analgesia in mice. Life Sci 1991;48:1827–35.

Takemori AE, Schwartz MM, Portoghese PS. Suppression by nor-binaltorphimine of kappa opioid-mediated diuresis in rats. J Pharmacol Exp Ther 1988;247:971–4.

Vivian JA, DeYoung MB, Sumpter TL, Traynor JR, Lewis JW, Woods JH. 6-Opioid receptor effects of butorphanol in rhesus monkeys. J Pharmacol Exp Ther 1999;290:259–65.

Walker E, Butelman E, de Costa B, Woods J. Opioid thermal antinociception in rhesus monkeys: receptor mechanisms and temperature dependency. J Pharmacol Exp Ther 1993;267:280–6.

Walker DJ, Zacny JP, Galva KE, Lichtor JL. Subjective, psychomotor and physiological effects of cumulative doses of mixed-action opioids in healthy volunteers. Psychopharmacology 2001;155:362–71.

Wongchanapai W, Tsang BK, He Z, Ho IK. Differential involvement of opioid receptors in intrathecal butorphanol-induced analgesia: compared to morphine. Pharmacol Biochem Behav 1998;59:723–7.

Zacny JP, Lichtor JL, Thapar P, Coalson DW, Flemming D, Thompson WK. Comparing the subjective, psychomotor and physiological effects of

intravenous butorphanol and morphine in healthy volunteers. J Pharmacol Exp Ther 1994;270:579–88.

Zacny JP, Conley K, Marks S. Comparing the subjective, psychomotor and physiological effects of intravenous nalbuphine and morphine in healthy volunteers. J Pharmacol Exp Ther 1997;280:1159–69.

Zacny JP, Hill JL, Black ML, Sadeghi P. Comparing the subjective, psychomotor and physiological effects of intravenous pentazocine and morphine in normal volunteers. J Pharmacol Exp Ther 1998;286:1197–207.

Zimmerman D, Leander J, Reel J, Hynes M. Use of β-funaltrexamine to determine mu opioid receptor involvement in the analgesic activity of various opioid ligands. J Pharmacol Exp Ther 1987;241:374–8.