Opioid Antinociception in Ovariectomized Monkeys: Comparison with Antinociception in Males and Effects of Estradiol Replacement

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ABSTRACT
Baseline nociception and opioid antinociception were compared in male and ovariectomized female rhesus monkeys. Females were studied without estradiol replacement or during treatment with estradiol benzoate at doses (0.002 and 0.01 mg/kg/day) designed to mimic 17β-estradiol blood levels observed during different phases of the menstrual cycle and during pregnancy. Baseline sensitivity to thermal stimuli (42–54°C) was similar in male and ovariectomized female monkeys. The antinociceptive effects of the μ-opioid agonist fentanyl, morphine, butorphanol, and nalbuphine were examined at 50 and 54°C. There were no sex-related differences in the antinociceptive effects of the high-efficacy μ opioid agonist fentanyl; however, the lower-efficacy μ agonists morphine, butorphanol, and nalbuphine produced greater antinociceptive effects in males than in untreated ovariectomized females. Because butorphanol and nalbuphine have low selectivity for μ versus κ receptors and may produce κ-agonist effects under some conditions, the high-efficacy, κ-selective agonist U50,488 was also studied. U50,488 also produced greater antinociceptive effects in males. Treatment with estradiol benzoate tended to enhance opioid antinociception in the ovariectomized females; however, this effect was significant only for butorphanol and U50,488 during treatment with the highest dose of estradiol benzoate. These findings suggest that opioid agonists usually produce greater antinociception in male monkeys than in females, and the magnitude of these sex-related differences may be inversely related to efficacy at μ receptors or selectivity for μ versus κ receptors. Estradiol appears to have little effect on μ-opioid antinociception in primates but may enhance the antinociceptive effects of κ agonists.

Accumulating evidence from preclinical studies in rodents suggests that there are sex-related differences in the antinociceptive effects of opioid agonists. Most of these studies were conducted with the prototype μ-opioid agonist morphine, and morphine was either more potent or produced greater antinociceptive effects in males than in females in nociceptive assays with thermal, chemical, and electrical noxious stimuli (Kavaliers and Innes, 1987; Baamonde et al., 1989; Kepler et al., 1989; Candido et al., 1992; Islam et al., 1993; Cicero et al., 1996; Craft et al., 1996; Boyer et al., 1998). Male rats and mice were also more sensitive than females to the antinociceptive effects of the other μ agonists, [d-Ala², N-MePhe⁴, Gly⁵-ol]-enkephalin (DAMGO) and alfentanil (Kepler et al., 1991; Cicero et al., 1997), the κ agonist U50,488 (Kavaliers and Innes, 1987), and the δ agonists [d-Pen²⁵]-enkephalin and deltorphin (Bartok and Craft, 1997). Stress-induced analgesia that is thought to be mediated by endogenous opioid systems (e.g., analgesia induced by intermittent cold water swim stress) also produced greater antinociception in males than in females (Bodnar et al., 1988). Sex-related differences in opioid antinociception have not always been observed, and such factors as the type of opioid, the type of antinociceptive assay, and the age of the subject may all be important determinants of drug effects (Kepler et al., 1991; Islam et al., 1993; Bartok and Craft, 1997). Taken together, however, these findings suggest that under many conditions, opioids produce greater antinociceptive effects in male rodents than in females.

The mechanisms underlying sex-related differences in opioid antinociception in rodents are unknown. Importantly, pharmacokinetic factors cannot account for differences in the effects of morphine in male and female rats (Craft et al., 1996; Cicero et al., 1997). Rather, sex-related differences in morphine antinociception may involve pharmacodynamic factors such as receptor density, receptor affinity for morphine, or the intra- or intercellular consequences of morphine binding to opioid receptors. Gonadal hormones may play a role in the development and maintenance of any sexually divergent biological substrates that mediate opioid antinociception. For example, opioid antinociception varied during the estrous cycle in female rats, and the greatest sensitivity

ABBREVIATIONS: DAMGO, [d-Ala², N-MePhe⁴, Gly⁵-ol]-enkephalin; % MPE, percent maximum possible effect.
to opioids occurred at times when estradiol levels were high (Banerjee et al., 1983; Kepler et al., 1989). Moreover, gonadectomy in adult male and female rats decreased the antinociceptive effects of both morphine and stress in some studies (Banerjee et al., 1983; Bodnar et al., 1988; Ryan and Maier, 1988; Kepler et al., 1989), although opioid antinociception was not affected by gonadectomy in other studies (Kepler et al., 1991; Cicero et al., 1996). Steroid replacement with testosterone or estradiol was also found to reinstate antinociception in gonadectomized male and female rats under some conditions (Banerjee et al., 1983; Bodnar et al., 1988, Ryan and Maier, 1988). Finally, late pregnancy and parturition are associated with an endogenous opioid-mediated maternal antinociception, and this pregnancy-related antinociception can be mimicked in ovariectomized rats by simulation of pregnancy profiles of estradiol and progesterone (Gintzler, 1980; Dawson-Basoa and Gintzler, 1993). These results suggest that gonadal hormones may be important modulators of opioidergic systems in adult rodents and may contribute to sex-related differences in the antinociceptive effects of opioids.

In contrast to the numerous studies that examined sex-related differences in opioid antinociception in rodents, there are no published reports that compared the antinociceptive effects of opioids in male and female nonhuman primates. However, the distribution of different opioid receptor types in human brain is more similar to that found in nonhuman primates than in rodents (Mansour et al., 1988). In addition, the reproductive systems and sex-related hormonal patterns in humans more closely resemble those found in nonhuman primates than in rodents (Knobil and Hotchkiss, 1988). Finally, sex-related differences in the analgesic effects of some opioids have also been reported recently in clinical studies (Gear et al., 1996a,b). Consequently, studies in primates may be especially important to further evaluate the generality and clinical relevance of interactions between opioids and gonadal hormones.

Accordingly, the purpose of the present study was to examine the antinociceptive effects of a range of opioid agonists in male and female rhesus monkeys. Female monkeys were ovariectomized before testing to eliminate hormonal fluctuations associated with the menstrual cycle and to permit experimental control of gonadal hormone levels. In particular, the effects of estradiol replacement were examined, because previous studies in rodents suggest that estrogens may modulate opioid receptor systems (Martini et al., 1989; Weiland and Wise, 1990; Maggi et al., 1991) and opioid antinociception (Bodnar et al., 1988; Ryan and Maier, 1988). The opioids selected for study included fentanyl, morphine, butorphanol, nalbuphine, and U50,488. The agonist effects of fentanyl, morphine, butorphanol, and nalbuphine are mediated primarily by μ-opioid receptors in rhesus monkeys (Negus et al., 1993; Gerak et al., 1994; Butelman et al., 1995). However, these compounds differ in their relative efficacy at μ receptors, with fentanyl displaying high efficacy, morphine intermediate efficacy, and butorphanol and nalbuphine low efficacy at μ receptors (Gatch et al., 1995; Emmerson et al., 1996; Butelman et al., 1998). These compounds also differ in their selectivity for μ receptors, and butorphanol and nalbuphine are relatively nonselective for μ versus κ-opioid receptors (Emmerson et al., 1994; Butelman et al., 1995, 1998). Although butorphanol and nalbuphine appear to possess low efficacy at μ receptors (Dykstra, 1990; Gerak et al., 1994; Butelman et al., 1995; Zhu et al., 1997), they produce diuretic effects in rodents (Leander, 1983) and subjective effects in humans (Gear et al., 1996b) that may be mediated by κ receptors. Consequently, we also examined the selective, high-efficacy κ-opioid agonist U50,488 (France et al., 1994; Zhu et al., 1997) for comparison to the μ agonists. All opioids were evaluated with a warm-water tail-withdrawal procedure that has been used extensively to examine the antinociceptive effects of opioids in rhesus monkeys (France et al., 1994; Gerak et al., 1994; Butelman et al., 1995; Gatch et al., 1995).

**Materials and Methods**

**Subjects**

The subjects were four intact male and four ovariectomized female rhesus monkeys (*Macaca mulatta*). Females had been ovariectomized for at least 2 months before the beginning of these studies to permit stabilization of anterior pituitary and ovarian hormone levels. All monkeys had experimental histories involving the administration of stimulant and/or opioid compounds. Monkeys weighed 5.8 to 10.5 kg and were maintained on a diet of fresh fruit, vegetables, and Lab Diet Jumbo Monkey biscuits (PMI Feeds, Inc., St. Louis, MO). Water was continuously available, and a 12-h light/dark cycle was in effect (lights on from 7:00 AM to 7:00 PM).

Animal maintenance and research were conducted in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was monitored periodically by consulting veterinarians. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Monkeys also had access to puzzle feeders, mirrors, and chew toys to provide environmental enrichment.

**Estradiol Replacement in Ovariectomized Monkeys**

Ovariectomized monkeys were studied in the absence of estradiol replacement and during treatment with 0.002 and 0.01 mg/kg/day estradiol benzoate (E$_2$β). These doses of E$_2$β were based on preliminary dose-ranging studies and were intended to mimic physiological estradiol levels observed during the mid-follicular and mid-luteal phases of the menstrual cycle (approximately 50–100 pg/ml), and the periovulatory phase of the menstrual cycle and the middle stages of pregnancy (approximately 300 pg/ml) (Atkinson et al., 1975; Knobil and Hotchkiss, 1988). During E$_2$β treatment, a dose of E$_2$β in sesame oil vehicle was administered i.m. at approximately 10:00 AM daily for up to 4 weeks. To ensure stable estradiol levels, monkeys were treated with E$_2$β for at least 7 days before studies were conducted. Doses of E$_2$β were studied in an irregular order across monkeys. At the conclusion of each E$_2$β treatment, there was a washout phase that lasted at least 1 month, and the next treatment was initiated only when estradiol levels had returned to baseline levels (defined as less than 20 pg/ml).

**Estradiol Analysis**

Blood samples were drawn weekly before, during, and after E$_2$β treatment, and plasma concentrations of 17β-estradiol were measured in duplicate by a direct, double-antibody radioimmunoassay with a kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). In a modification to the protocol, the plasma samples were extracted and reconstituted in zero standard before the assay. Results are expressed in picograms per milliliter. The assay sensitivity was 6.3 pg/ml, and the intra- and interassay c.v.s were 6.5 and 11.1%, respectively.
Assay of Thermal Nociception

Behavioral Procedure. Studies of thermal nociception were conducted no more than twice a week, and each experiment began at approximately 1:00 PM, 3 h after administration of E$_2$B treatments. Each monkey was seated in an acrylic restraint chair. The bottom 10 cm of the monkey’s shaved tail was immersed in a thermal container of warm water, and tail-withdrawal latencies were measured from water heated to four different temperatures (42, 46, 50, and 54°C). An Apple IIe microcomputer was used to measure and record tail-withdrawal latencies. If the subject did not withdraw its tail within 20 s, the timer was stopped, and a tail-withdrawal latency of 20 s was assigned to that measurement. Tail-withdrawal latencies from all four water temperatures were measured every 30 min for up to 3 h, and temperatures were presented in a pseudorandom sequence across cycles. During any one cycle of measurements, the order in which temperatures were presented was the same across subjects.

Pharmacological Procedure. Before the first test cycle, baseline tail-withdrawal latencies from 42, 46, 50, and 54°C water were determined. For cumulative dosing experiments with test compounds, a single drug dose was administered every 30 min, and each injection increased the total dose by $\frac{1}{4}$ or $\frac{1}{2}$ log increments. Fifteen minutes after each injection, tail-withdrawal latencies were redetermined as described above.

The opioids studied were fentanyl, morphine, butorphanol, nalbuphine, and U50,488. Fentanyl, morphine, butorphanol, and nalbuphine produce agonist effects that are mediated primarily by $\mu$-opioid receptors in rhesus monkeys, and these four compounds display a range of efficacies at $\mu$ receptors (Gerak et al., 1994; Butelman et al., 1995; Gatch et al., 1995; Emmerson et al., 1996). Specifically, fentanyl has relatively high efficacy, morphine has intermediate efficacy, and butorphanol and nalbuphine have low efficacy at $\mu$-opioid receptors. The high-efficacy, $\kappa$-selective agonist U50,488 (France et al., 1994; Zhu et al., 1997) was also studied for comparison with $\mu$ agonists. Opioid agonists were studied in an irregular order in males and in females during the different E$_2$B treatment conditions.

Assay of Morphine Pharmacokinetics

To determine whether there were differences in morphine pharmacokinetics in males and ovariectomized females with and without E$_2$B replacement, blood samples were collected for morphine analysis in separate studies. Monkeys were sedated with ketamine (5 mg/kg), and a Surflo i.v. catheter (Terumo Medical Corp., Elkton, MD) for blood sample collection was implanted in one saphenous vein. Monkeys were then placed in acrylic primate restraint chairs for at least 1 h and until recovery from ketamine sedation was complete. After the collection of two baseline blood samples, a single dose of 10 mg/kg morphine was administered i.m. This dose and route of administration were selected to permit pharmacokinetic evaluation of a dose of morphine that produced different levels of antinociception in male and female monkeys (see below). After morphine administration, blood samples were collected at 7.5-min intervals for the first hour, at 15-min intervals for the second hour, and at 30-min intervals for the third hour. Only three of the four male monkeys were used in studies of morphine pharmacokinetics, because a patent i.v. catheter for blood collection could not be maintained in the fourth male monkey.

Plasma morphine concentrations were measured in duplicate by a direct, solid phase radioimmunoassay method with a kit purchased from Diagnostic Products Corp. (Los Angeles, CA). Results are expressed in nanograms per milliliter. The assay sensitivity was 0.5 ng/ml, and the intra- and interassay c.v.s were 2.5 and 5.1%, respectively.

Data Analysis

Tail-withdrawal latency data were converted to percent maximum possible effect (% MPE) by using the equation $\left[\frac{\text{Test Latency} - \text{Baseline Latency}}{\text{Baseline Latency}}\times (20 - \text{Baseline Latency})\right] \times 100$, where Test Latency was the tail-withdrawal latency in seconds at a given temperature measured during a test cycle. Baseline Latency was the baseline tail-withdrawal latency in seconds observed at that temperature at the beginning of the session, and 20 was the maximum number of seconds that could be assigned to any tail-withdrawal latency measurement. Mean values for % MPE ($\pm$S.E.M.) were then plotted as a function of drug dose.

Baseline tail-withdrawal latencies, opioid effects on % MPE at 50 and 54°C, and plasma morphine levels were compared between males and ovariectomized females (in the absence of estrogen replacement) by using two-factor ANOVAs, with sex as a between-subjects factor, and temperature, drug dose, or time after drug injection as a within-subject factor. Two-factor ANOVAs were also conducted on data from ovariectomized females during treatment with different doses of estradiol, with dose of estradiol as one within-subject factor, and temperature, drug dose, or time after drug injection as a second within-subject factor. Serum levels of 17β-estradiol in females during treatment with different doses of E$_2$B were compared by using a one-factor ANOVA, with dose of estradiol as a single, within-subject factor. For all comparisons, a significant ANOVA was followed by individual means comparisons that used simple effects. The criterion for significance was set at $p < .05$. All statistical analyses were conducted with the CLR Analysis of Variance Program for the Apple Macintosh (Clear Lake Research, Houston, TX).

Additionally, estimates of the pharmacokinetic parameters peak plasma morphine concentrations ($C_{\text{max}}$), time to peak plasma morphine concentrations ($T_{\text{max}}$), and area under the concentration-time curve (AUC) for morphine were obtained directly from a nonlinear regression estimation software program based on the Manual of Pharmacologic Calculations with Computer Programs with PHARM/PCS Version 4.2 (Microcomputer Specialist MCS, Philadelphia, PA). Plasma drug concentrations were fitted to a single-dose, one-compartment model with bolus input, first order output, and elimination. Area under the curve determinations were estimated by the linear trapezoidal rule. Pharmacokinetic parameters were analyzed by one-factor ANOVAs, with either sex as a single-between-subjects factor, or E$_2$B dose as a within-subject factor.

Drugs

Fentanyl hydrochloride and morphine sulfate (provided by the National Institute on Drug Abuse, Bethesda, MD), and nalbuphine hydrochloride and (±)-trans-U50,488 methanesulfonate (Research Biochemicals International, Natick, MA) were dissolved in distilled water. Butorphanol tartrate was used as the commercially available Torbutrol solution (Fort Dodge Animal Health, Overland Park, KS). Estradiol benzoate (Sigma Chemical Co., St. Louis, MO) was dissolved in sesame oil. Doses are expressed as milligrams per kilogram of the salt form of the compound. All drugs were administered i.m. in the thigh.

Results

Effects of E$_2$B Treatment on 17β-Estradiol Levels in Ovariectomized Monkeys. Treatment with E$_2$B produced a dose-dependent increase in serum levels of 17β-estradiol ($p < .001$). In the absence of E$_2$B treatment, levels of 17β-estradiol were near the threshold of detection of the assay (6.61 ± 0.51 pg/ml). Treatment with 0.002 and 0.01 mg/kg/day E$_2$B produced 17β-estradiol levels of 78.74 (±6.61) and 283 (±20.61) pg/ml, respectively. Both E$_2$B treatments produced 17β-estradiol levels significantly higher than those observed in the absence of treatment ($p < .05$).

Baseline Thermal Nociception. Figure 1 shows baseline tail-withdrawal latencies from 42, 46, 50, and 54°C water in males and in ovariectomized females during treatment with
different doses of E$_2$β. In all monkeys under all conditions, tail-withdrawal latencies decreased as a function of increasing water temperature. Monkeys never withdrew their tails from 42°C water. Mean tail-withdrawal latencies from 46°C water ranged between 8 and 15 s, and tail-withdrawal latencies from 50 and 54°C water were consistently <2 s.

Females were slightly more sensitive to thermal noxious stimuli than were ovariectomized females in the absence of E$_2$β treatment (Fig. 1, left). Comparison of baseline nociceptive response in males and ovariectomized females indicated that males showed a significantly lower tail-withdrawal latency than did females from 46°C water ($p = .024$). However, this difference was small, and tail-withdrawal latencies at other temperatures were similar in males and ovariectomized females.

E$_2$β treatment produced a slight increase in the sensitivity of ovariectomized females to thermal stimuli (Fig. 2, right). Tail-withdrawal latencies at 46°C were significantly lower during both E$_2$β treatments than during the absence of treatment ($p = .037$). However, these differences were small, and tail-withdrawal latencies at other temperatures were similar across E$_2$β treatment conditions.

**Antinociceptive Effects of μ-Opioid Agonists.** Figure 2 compares the antinociceptive effects of the μ agonists fentanyl, morphine, butorphanol, and nalbuphine at 50 and 54°C in males and in ovariectomized females in the absence of E$_2$β replacement. ANOVA results are summarized in Table 1. All four μ agonists produced dose-dependent antinociceptive effects. However, the magnitude of antinociception differed across compounds. Across the dose range tested, fentanyl and morphine both produced maximal or near-maximal effects in all monkeys tested at 50°C and submaximal effects at 54°C. Higher doses of fentanyl and morphine were
not examined in this study to avoid toxic effects such as severe respiratory depression; however, in previous studies, we found that both compounds produced greater antinociceptive effects at higher doses (Gatch et al., 1995). Butorphanol and nalbuphine failed to produce maximal effects in all monkeys at 50°C, and both compounds produced submaximal effects at 54°C. In addition, the antinociceptive effects of butorphanol and nalbuphine appeared to plateau at the highest doses tested, which suggests that even higher doses would not have produced greater antinociceptive effects. Butorphanol generally produced greater maximal effects than nalbuphine.

There was a tendency for μ agonists to be more potent and/or more effective in males than in females. This difference did not attain statistical significance for the high-efficacy μ agonist fentanyl. However, significant differences were observed for morphine, butorphanol, and nalbuphine (see Table 1), and in each case in which significant sex-related differences were observed, the μ agonists produced greater antinociceptive effects in males than in females. Specifically, morphine (3.2 mg/kg) and butorphanol (0.01 and 0.032 mg/kg) produced greater antinociception in males than in ovariectomized females at 50°C (Fig. 2, top). Sex-related differences were even more pronounced at the higher temperature of 54°C. At this temperature, morphine (10 mg/kg), butorphanol (0.01–0.32 mg/kg), and nalbuphine (10 and 18 mg/kg) produced greater antinociception in males than in ovariectomized females (Fig. 2, bottom).

Figure 3 shows the antinociceptive effects of the same μ agonists in females during treatment with different doses of E₂β, and ANOVA results are summarized in Table 1. All four μ agonists produced dose-dependent antinociceptive effects. There was also a tendency for E₂β treatment to increase the potency and/or maximal effects of the μ agonists. However, this effect attained statistical significance only for butorphanol at 54°C. Specifically, a dose of 0.32 mg/kg butorphanol produced significantly greater antinociception during treatment with 0.01 mg/kg/day E₂β than in the absence of E₂β treatment.

Pharmacokinetics of Morphine. Figure 4 shows plasma levels of morphine at various times after administration of 10 mg/kg morphine (i.m.). The left panel compares males and ovariectomized females during the absence of E₂β treatment. The right panel compares females in the absence of E₂β treatment and during treatment with 0.01 mg/kg/day E₂β. Pharmacokinetic parameters estimated from these curves are shown in Table 2. Under all conditions, plasma morphine levels rose rapidly and usually peaked at about 2200 to 2600 ng/ml during the second or third sample (i.e., after 15 or 22.5 min). Morphine levels then decreased during the remainder of the sampling period and ranged between 500 and 1000 ng/ml after 3 h. Although minor differences in plasma morphine levels were observed, there were no significant differences between males and ovariectomized females without E₂β treatment at any time after morphine injection, and there were no significant differences in Cₘₐₓ, Tₘₐₓ, or AUC. In the ovariectomized females, there were no significant differences in plasma morphine levels or estimated pharmacokinetic parameters during the absence of E₂β treatment or during treatment with 0.01 mg/kg/day E₂β.

Antinociceptive Effects of the κ-Opioid Agonist U50,488. Figure 5 (left) compares the antinociceptive effects of U50,488 in males and ovariectomized females in the absence of E₂β treatment. ANOVA results are summarized in Table 1. U50,488 produced dose-dependent antinociceptive effects in both males and ovariectomized females; however, U50,488 produced greater antinociceptive effects in males. Specifically, a dose of 0.32 mg/kg U50,488 produced greater antinociception in males than in ovariectomized females at 50°C, and there was a tendency for U50,488 to produce greater maximal effects in males than in females at 54°C. It is also important to note that comparisons could only be made across the dose range of 0.1 to 1.0 mg/kg U50,488. A dose of 1.0 mg/kg U50,488 produced greater than 90% MPE at both 50 and 54°C in the males, and higher doses were not tested due to the emergence of toxic effects including sedation and polymyoconus. U50,488 produced less overt toxicity in the ovariectomized females, and doses up to 3.2 mg/kg were tested safely. However, even at doses up to 3.2 mg/kg, U50,488 never produced more than 42% MPE at 54°C in the untreated females.

Figure 5 (right) compares U50,488-induced antinociception in ovariectomized female monkeys during treatment with different doses of E₂β. There was a tendency for E₂β to

TABLE 1
Summary of two-way ANOVA results for opioid antinociception data (Figs. 3, 4, and 6)
The first three columns show p values for the opioid agonist dose main effect (within-subject variable), gender main effect (between-subject variable), and dose × gender interaction for comparisons between males and ovariectomized females in the absence of E₂β (Fig. 3 and left panels of Fig. 6). The second three columns show p values for the opioid agonist dose main effect (within-subject variable), E₂β dose main effect (within-subject variable), and opioid dose × E₂β dose interaction for ovariectomized females during treatment with different doses of E₂β (Fig. 4 and right panels of Fig. 6). Significant ANOVAs (i.e., p < 0.05) are shown in bold print.

<table>
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<tr>
<th>Dose</th>
<th>Gender</th>
<th>Dose × Gender</th>
<th>Dose</th>
<th>E₂β</th>
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<td>0.012</td>
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<td></td>
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<td>0.040</td>
<td>0.029</td>
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</tr>
<tr>
<td>Butorphanol</td>
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<td>0.220</td>
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<tr>
<td></td>
<td>54°C</td>
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<td>0.004</td>
<td>0.033</td>
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increase the potency and/or maximal effect of U50,488. E2 treatment did not significantly alter the antinociceptive effects of U50,488 at the lower temperature of 50°C. However, at 54°C, the antinociceptive effects of 1.0 to 3.2 mg/kg E2 treatment were significantly greater during 0.01 mg/kg/day E2 treatment than in the absence of E2 treatment. Treatment with E2 did not appear to alter the sedative or polymyoclonic effects of U50,488 in ovariectomized females.

**Discussion**

**Baseline Nociception.** Our finding that tail-withdrawal latencies were inversely related to water temperature across a range of 42–54°C is consistent with previous studies in rhesus monkeys (Gatch et al., 1995). Moreover, the temperature-response function was similar in males and ovariectomized females. However, male monkeys were slightly more sensitive than ovariectomized females without E2 treatment to an intermediate-intensity thermal noxious stimulus (46°C), and treatment with estradiol produced a small increase in thermal sensitivity in the females.

The biological relevance of these small differences in baseline thermal nociception remains to be determined. Most previous studies that used thermal noxious stimuli in rodents did not detect a difference in baseline nociception between males and females (Kavaliers and Innes, 1987; Candido et al., 1992; Cicero et al., 1996; Craft et al., 1996). These studies typically used relatively high-intensity stimuli designed to minimize intersubject variability in response latencies. When high-intensity stimuli (i.e., 50 and 54°C water) were used in the present study, response latencies in males and ovariectomized females were also similar. Moreover, most studies conducted in rodents have not controlled for estrous cycle phase in females, and as a result, possible fluctuations in nociception associated with cycle variations in anterior pituitary and gonadal hormone levels (Kepler et al., 1989) may have been obscured. However, sex-related differences in thermal nociception have occasionally been described. For example, in agreement with the present study, female rats had longer response latencies than did males in a tail-flick test (Islam et al., 1993) and a hot-plate test (Bartok and Craft, 1997). On the other hand, female rats were more sensitive than males when shock (Kepler et al., 1991) or formalin injection in the paw (Aloisi et al., 1994) was used as a noxious stimulus, and studies in humans suggest that women are usually more sensitive to painful stimuli than men (for review, see Fillingim and Maixner, 1995). Overall, any sex-related differences in thermal nociception have been small. In the present study, the antinociceptive effects of opioids were examined only for high-intensity thermal stimuli, because male and ovariectomized female monkeys had similar and stable baseline tail-withdrawal latencies under these conditions.
Sex-Related Differences in Opioid Antinociception in Nonhuman Primates: Comparison to Findings in Rodents and Humans.

Previous studies conducted in rodents usually found that opioid agonists were more potent or produced greater antinociceptive effects in males than in females (Kavaliers and Innes, 1987; Baamonde et al., 1989; Kepler et al., 1989; Candido et al., 1992; Islam et al., 1993; Cicero et al., 1996, 1997; Craft et al., 1996). The present study provides the first evidence to suggest that there are also sex-related differences in the antinociceptive effects of opioids in nonhuman primates. As in rodents, both μ and κ agonists tended to produce greater effects in male rhesus monkeys than in ovariectomized females. Moreover, a dose of 10 mg/kg morphine, which produced greater antinociception in males, produced similar plasma levels of morphine in male and ovariectomized female monkeys. One limitation of these pharmacokinetic studies was that only one dose of morphine was tested, and sex-related differences in morphine pharmacokinetics might be revealed at other doses. In addition, sex-related differences in brain levels of morphine may exist.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>OVX Females-No Eβ</th>
<th>OVX Females + 0.01 mg/kg/day Eβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>2,628 (277)</td>
<td>2,510 (493)</td>
<td>2,230 (216)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>25.0 (10.9)</td>
<td>15.9 (5.3)</td>
<td>22.5 (9.2)</td>
</tr>
<tr>
<td>AUC (ng/ml/ min)</td>
<td>253,749 (16,794)</td>
<td>214,454 (25,586)</td>
<td>188,646 (16,407)</td>
</tr>
</tbody>
</table>

Fig. 4. Plasma morphine levels after i.m. injection of 10 mg/kg morphine in male monkeys and in ovariectomized female monkeys during daily treatment with Eβ. Abscissae, time after morphine injection in minutes. Ordinates, plasma morphine levels in nanograms per milliliter. The left panel compares data from male monkeys and from ovariectomized females in the absence of Eβ treatment. The right panel compares data in female monkeys during treatment with different doses of Eβ. All points show mean data (±S.E.M.) from four monkeys, except for the male monkeys in the left panel, which shows data from only three monkeys. Points to the left of time 0 show baseline data collected before morphine injection.

**Fig. 5.** Antinociceptive effects of the κ-opioid agonist U50,488 in male monkeys and in ovariectomized female monkeys during treatment with different doses of Eβ. Abscissae, dose of U50,488 in milligrams per kilogram. Ordinates, % MPE. Top panels show data obtained with 50°C water, and bottom panels show data obtained with 54°C water. The left panel compares data from male monkeys and from ovariectomized female monkeys in the absence of Eβ treatment. The right panel compares data in female monkeys during treatment with different doses of Eβ. All points show mean data (±S.E.M.) from four monkeys. *, a significant difference from the OVX Females-No Eβ data.
Despite similar plasma levels of morphine (Craft et al., 1996). However, the results of the present study agree with results obtained in rodents (Craft et al., 1996; Cicero et al., 1997) to suggest that the sex-related differences in morphine antinociception do not appear to result solely from differences in morphine pharmacokinetics.

Additional research will be required to assess the range of conditions under which opioid antinociception is influenced by the sex of the subject. The importance of such research is suggested by two recent clinical studies that compared the analgesic effects of the opioids pentazocine, butorphanol, and nalbuphine in men and women who had undergone dental surgery. In contrast to our findings in monkeys and the findings of most studies in rodents, all three opioids were reported to produce greater analgesic effects in women than in men (Gear et al., 1996a,b). The reason for this discrepancy is not known; however, there were many procedural differences that may have contributed to the different results. For example, these clinical and preclinical studies measured different aspects of pain (subjective ratings of pain versus latency to nociceptive withdrawal responses) elicited by different events (postsurgical inflammation versus acute noxious stimuli).

Role of Opioid Agonist Efficacy at \(\mu\)-Opioid Receptors as a Determinant of Sex-Related Differences in Antinociception. The effects of the high-efficacy, high-selectivity \(\mu\) agonist fentanyl were not significantly different in males and ovariectomized females; however, sex-related differences were observed with morphine, butorphanol, and nalbuphine, which have lower efficacy at \(\mu\) receptors than fentanyl (Emmerson et al., 1994, 1996; Gatch et al., 1995; Butelman et al., 1998). Indeed, the most pronounced sex-related differences in antinociception were observed with butorphanol and nalbuphine, the agonists with the lowest efficacy for \(\mu\) receptors. These findings suggest that the magnitude of these differences may be inversely related to \(\mu\) agonist efficacy.

It is well established that the effects of low-efficacy agonists are more sensitive than those of high-efficacy agonists to changes in the number of available receptors or the efficiency of receptor/effector coupling (Kenakin, 1993). For example, administration of an irreversible \(\mu\)-opioid receptor antagonist, which decreases the total number of available receptors, has been shown to produce a greater antagonism of antinociception produced by low-efficacy \(\mu\) agonists than by high-efficacy \(\mu\) agonists (Walker et al., 1995). Consequently, the greater sex-related differences observed with low-efficacy \(\mu\) agonists in the present study are suggestive of sex-related differences in \(\mu\)-opioid receptor systems that subserve antinociception. Specifically, it is possible that the ovariectomized females in this study either had fewer \(\mu\) opioid receptors than males or had less efficient mechanisms for transducing drug-receptor interactions into an antinociceptive response. Previous studies that compared opioid antinociception in male and female rodents did not explicitly manipulate \(\mu\) agonist efficacy. However, the magnitude of sex-related differences in opioid antinociception has been found to vary across \(\mu\) agonists in rodents under some conditions, and these findings may also be related to \(\mu\) agonist efficacy. For example, in agreement with the present study, nalbuphine and morphine produced greater antinociceptive effects in male rats than in females in a 52°C hot-plate test, but the effects of fentanyl were similar in males and females (Craft et al., 1996; Bartok and Craft, 1997). Similarly, sex-related differences in opioid antinociception were observed with morphine but not with the higher efficacy peptidic \(\mu\) agonist DAMGO in a shock-induced jump test (Kepler et al., 1989, 1991).

Role of Opioid Agonist Selectivity as a Determinant of Sex-Related Differences in Antinociception. The differential selectivity of fentanyl, morphine, butorphanol, and nalbuphine for \(\mu\) versus non-\(\mu\) receptors, and in particular \(\mu\)-versus \(\kappa\)-opioid receptors, may also have contributed to the results of this study. Both butorphanol and nalbuphine bind to \(\kappa\) receptors with affinities only slightly lower than their affinities for \(\mu\) receptors (Butelman et al., 1995, 1998). Both compounds have very low efficacy at \(\kappa\) receptors and either do not produce detectable \(\kappa\)-mediated effects or function primarily as \(\kappa\)-opioid antagonists in monkeys (Dykstra, 1990; Gerak et al., 1994; Butelman et al., 1995). Thus, it is unlikely that \(\kappa\) receptors played an important role in mediating the antinociceptive effects of butorphanol or nalbuphine. However, weak \(\kappa\) agonist effects (e.g., diuresis) have been observed under some conditions in rodents (Leander, 1983). In addition, butorphanol and nalbuphine produce some subjective effects in humans that are similar to the effects of prototype \(\kappa\)-opioid agonists, which suggests that at least some effects of butorphanol and nalbuphine in humans may be mediated by \(\kappa\) receptors (Jasinski and Mansky, 1972; Preston et al., 1989; Gear et al., 1996b). In the present study, we found that the high-efficacy \(\kappa\)-opioid agonist U50,488 produced sex-related differences in antinociception similar to those produced by butorphanol and nalbuphine. These results confirm previous findings of sex-related differences in U50,488-mediated antinociception in rodents (Kavaliers and Innes, 1987) and suggest that \(\kappa\) agonists, like \(\mu\) agonists, may produce greater antinociceptive effects in males than in females. As a result, it is possible that the sex-related differences in antinociception produced by butorphanol and nalbuphine may have resulted, at least in part, from sex-related differences in their effects at \(\kappa\)-opioid receptors.

Effects of Estradiol Treatment on Opioid Antinociception. There was a tendency for estradiol replacement to increase the antinociceptive effects of opioids. However, this effect achieved statistical significance only for butorphanol and U50,488 and only during treatment with the highest dose of estradiol, which produced high blood levels of estradiol similar to those observed during the periovulatory stage of the menstrual cycle and the middle stages of pregnancy (approximately 300 pg/ml) (Atkinson et al., 1975; Knobil and Hotchkiss, 1988). Overall, these results suggest that estradiol replacement over the dose range studied has little effect on antinociception produced by \(\mu\) agonists in ovariectomized monkeys, although estradiol may play a greater role in \(\kappa\) receptor-mediated antinociception.

The effects of estradiol replacement on opioid antinociception in gonadectomized rodents have also been inconsistent (Banerjee et al., 1983; Bodnar et al., 1988; Ryan and Maier, 1988). For example, estrogen was reported to produce a biphasic effect on an opioid-mediated form of stress-induced analgesia in ovariectomized rats, and intermediate doses of estrogen produced the most effective enhancement of stress-induced analgesia (Ryan and Maier, 1988). Moreover, treatment with estradiol and progesterone to simulate pregnancy...
levels of these hormones in rats enhanced the antinociceptive effects of the κ agonist U50,488 but not the μ agonist sufentanil (Dawson-Basoa and Gintzler, 1996). However, treatment with pregnancy levels of estradiol alone may not be sufficient to produce these effects in rats (Dawson-Basoa and Gintzler, 1993). In addition, other studies found that estradiol treatment either did not affect (Banerjee et al., 1983) or decreased (Berglund et al., 1988) morphine-induced antinociception in ovariec-tomized rats. Estrogens also produced variable effects on opioid receptor densities in ovariec-tomized rats (Martini et al., 1989; Weiland and Wise, 1990; Maggi et al., 1991). Thus, estrogens appear to modulate both opioid receptor populations and opioid-induced antinociception under some conditions, but the determinants of these estrogen-opioid interactions require further clarification.

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References


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