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Study of morphine-induced dependence in gonadectomized male and female mice

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ABSTRACT

In this study we evaluated the effects of sex difference and also sex hormones on the naloxone-precipitated morphine withdrawal in both orchidectomized (ORC) male and ovariectomized (OVX) female mice. Morphine (50, 50 and 75 mg/kg/day for 4 days, s.c.) was administered to animals and at 5th day naloxone (4 mg/kg, i.p.)-precipitated morphine withdrawal signs, jumpings and the percentage of weight loss, were measured. There was no significant alteration in withdrawal jumpings between male and female mice, though weight loss was significantly higher in male ones. Jumpings was significantly lower in both OVX and ORC mice and percentage of weight loss was significantly higher in OVX mice than corresponding non-operated or sham animals. In OVX mice, E_2V (10 mg/kg, s.c.) increased number of jumpings and decreased percentage of weight loss. Progesterone (25 mg/kg, s.c.) had no effect on jumpings, whereas it decreased weight loss in OVX mice. Testosterone (2.5 mg/kg, s.c.) increased jumpings in ORC mice while it had no effect on percentage of weight loss. Our results demonstrated that sex hormones could play a role in the morphine withdrawal syndrome in both ORC male and OVX female mice.

1. Introduction

Animal studies have shown that males and females have different sensitivities to the effects of opioids. For example, male rats are more sensitive than females to the antinociceptive properties of morphine in several different pain models (Ali et al., 1995; Baamonde et al., 1989; Berkley, 1997; Cicero et al., 1996; Craft, 2003; Craft et al., 2004). Similar sex differences have been reported for morphine-induced sedation (Craft et al., 1999a; Stratmann and Craft, 1997), respiratory depression (Dahan and Kest, 2001; Kest et al., 1998; Pleym et al., 2003), urinary retention (Craft et al., 2000) and modulation of seizure susceptibility (Riazi et al., 2004) and locomotor activity (Craft et al., 2006; Li et al., 1990; Schnur and Barela, 1984). There is also several evidence demonstrating sex differences in the development of tolerance and dependence to opioids such as morphine (Craft et al., 2004; 1999b; Dahan et al., 2008). Additionally, it has been shown that sex differences in tolerance development could be explained by sex differences in morphine potency and when males and females receive the same mg/kg doses of morphine, males become more tolerant than females because they are more sensitive than females to the acute effects of morphine (Barrett et al., 2001). Sex hormones are probably the most investigated factors in the existing differences between males and females in response to opioids (Dahan et al., 2008). Some evidence exists for an interaction between sex hormones and opioid antinociceptive potency (Candido et al., 1992; Ji et al., 2007; Stoffel et al., 2003; Sumner et al., 2006). Moreover, the removal of sex steroids by gonadectomy affects the antinociceptive activity of morphine in some experimental paradigms (Banerjee et al., 1983; Bodnar et al., 1988; Cicero et al., 1996; Frye and Seliga, 2001; Negus and Mello, 2002; Romero et al., 1987).

Despite the aforementioned role of sex hormones in some of the properties of opioids, this issue has not been fully examined in the dependency to morphine. On the other hand, Reddy and Kulkarni (1997) demonstrated that chronic treatment of neurosteroids such as allopregnanolone, pregnenolone sulfate, dehydroepiandrosterone sulfate and progesterone during the induction of morphine dependency could affect the naloxone-precipitated withdrawal jumps. According to another observation, whereas chronic morphine decreased brain concentrations of pregnenolone, progesterone and pregnenolone sulfate, but not allopregnanolone, dihydroepiandrosterone and dihydroepiandosterone, naloxone-precipitated morphine withdrawal increased all of these steroid concentrations (Yan and Hou, 2004). Concas et al. (2006) has recently shown that acute treatment of morphine induced dose- and time-dependent increased in rat cerebrocortical and plasma concentrations of neurosteroids such as progesterone and pregnanolone. They also demonstrated that naloxone-precipitated morphine withdrawal also increased neurosteroid concentrations in rat brain and plasma. Another study by Ceccarelli et al. (2006) also revealed that opioid administration could alter the level of gonadal steroids such as estradiol and testosterone in both the central nervous system (CNS) and plasma of male rats. Considering the

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interactions between sex hormones and opioid system, it is possible that sex hormones might be involved in the dependency to morphine and morphine withdrawal syndrome.

In the present study, we first examined the differences in the naloxone-precipitated morphine withdrawal between male and female mice, evaluating whether there is any sex difference in dependency to morphine. Then we examined the effect of gonadectomy in this manner. Effects of sex hormones on the morphine withdrawal in both ovariectomized female and orchidectomized male mice were further evaluated.

2. Materials and methods

2.1. Animals

Female and male NMRI mice (Pasteur Institute) weighing 25–30 g were used throughout the study. The animals were housed in a temperature-controlled room (24±1 °C) on a 12-h light/dark cycle with free access to food and water. All experiments were carried out in the same room between 08:00 to 16:00 to minimize diurnal variations. Separate groups of animals were used for each test. All procedures were carried out in accordance with the institutional animal care and use committee (Department of Pharmacology, School of Medicine, TUMS) guidelines for animal care and use. All of the animal studies were also approved by a group from the Ethics Committee of Tehran University of Medical Sciences (TUMS) and experiments were performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Either of male and female animals was divided into 3 main groups: unoperated control, sham-operated and operated (OVX in female and ORC in male mice). Each experimental group consisted of 10 animals.

2.2. Chemicals

The following drugs were used in the study: morphine sulfate (Tolid-daru, Tehran, Iran), estradiol valerate (E_2V) and progesterone (Sigma; Poole, UK), testosterone enanthate (Tolid-daru, Tehran, Iran), and naloxone hydrochloride (Tolid-daru, Tehran, Iran). Morphine and naloxone were dissolved in physiologic saline solution. Estradiol valerate (E_2V), progesterone and testosterone were dissolved in olive oil. Doses used were chosen based on previous published studies and pilot experiments (Bassol et al., 2000; Dambisya and Lee, 1996; Nakazawa et al., 2006; Quirarte et al., 2007; Shirwalkar et al., 2007; Zarrindast et al., 2002).

2.3. Ovariectomy (OVX) and orchidectomy (ORC)

Anesthesia was induced by intraperitoneally injection of 50 mg/kg ketamine (Alfasan, Woerden, Holland). In female mice, after the onset of anesthesia, the lumbar dorsum was shaved, and the exposed skin prepared for aseptic surgery (a 10% povidene–iodine scrub followed by a sterile saline wipe). Surgery was performed according to the method described by Eddy (1986) with modification (Riazi et al., 2004). In brief, skin was opened with a 1- to 2-cm incision in the midline on the lumbar vertebral line. About 1 cm to each flank, parovarian fatty tissue was identified and pulled out through a small incision. The exposed ovary and associated oviduct were removed. Hemostasis, if needed, was achieved by hemostat pressure for 1 to 2 min. Then the skin incision was sutured (5-0 nonabsorbable). In sham-operated animals, the parovarian fatty tissues and ovaries were just retracted and were replaced.

In males, after proper anesthesia, a small 10-mm median incision was made through the skin at the tip of the scrotum. The cremaster muscles were opened with a small 7-mm incision. The testicular fat pads on both sides were pulled through the incision by using a blunt forceps. A single ligature was placed around the vas deferens and the blood vessels on each side, and the testicles were removed. Then the muscle layer was closed by using two resorbable 5-0 sutures, and the skin, with nonresorbable 4-0 sutures. The operated-on animals were tested 10–12 days after surgery. The mortality rate was <5%.

2.4. Induction of morphine dependence

The animals were rendered dependent on morphine using the method described previously (Zarrindast et al., 2002) with some modification. Morphine sulfate was injected subcutaneously three times daily at 08.00, 11.00 and 16.00 h at doses of 50, 50 and 75 mg/kg, respectively. The higher dose at the third daily injection was aimed to minimize any overnight withdrawal. Morphine administration was carried out 4 days for all groups of mice. A dose of 100 mg/kg of morphine sulphate was also injected on the test (fifth) day (2 h before naloxone injection). Additionally, a separate group of experimental animals were administered saline (instead of morphine) as a control group for further investigation of the effect of sex hormones on naloxone-withdrawal signs.

2.5. Naloxone-precipitated withdrawal

Two hours after the last dose of morphine on the fifth day, signs of abstinence were precipitated by intraperitoneally injection of 4 mg/kg of naloxone. Immediately after the injection of naloxone, the animals were placed individually in a platform (40 cm long, 25 cm wide and 45 cm high) and then the number of jumpings (withdrawal signs) was observed continuously for 30 min. The percentage loss of body weight at 1 h after naloxone administration was also measured.

2.6. Treatments

In experiment 1, for assessing the effects of the ovarian sex hormones on the withdrawal signs in female mice, OVX animals received separately single dose of either E_2V (10 mg/kg, s.c.) 48 h before the injection of naloxone. Single administration of 10 mg/kg E_2V could provide continuous levels of circulating estradiol for an extended period in rodents (Quirarte et al., 2007). In addition, 2 mg doses of E_2V along with a progesterone-like drug, given monthly, are the doses used in birth control drugs touted as effective for use by women of underdeveloped countries with special reference to Latin America (Bassol et al., 2000). Progesterone (25 mg/kg, s.c.) was also injected to a separate group of sham and OVX mice daily for 4 days during the development of morphine dependence before administration of the first dose of morphine each day. Control animals received vehicle (olive oil).

In experiment 2, for assessing the effects of the male sex hormone testosterone on the withdrawal signs in male mice, ORC animals received separately single dose of testosterone enanthate (2.5 mg/kg, i.p.) 48 h before the injection of naloxone. Control animals received vehicle.

2.7. Statistical analysis

All data are shown as the means±S.E.M. of value for corresponding parameters. Statistical comparison between groups in each experiment was done with one- or two-way analysis of variance (ANOVA) followed by post hoc Student–Newman–Keuls test. In a few cases in which only two groups were to be compared, Student's *t*-test was used. A *P* value less than 0.05 was considered the limit of significance.

3. Results

3.1. Sex differences in morphine withdrawal and effect of gonadectomy in this manner

Fig. 1 shows the effects of sex on the naloxone-precipitated withdrawal signs in intact mice and also effects of both ovariectomy