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Research Report

# Comparison of the antinociceptive effect of acute morphine in female and male Sprague–Dawley rats using the long-lasting mu-antagonist methocinnamox

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**Abstract**

Male rats are more sensitive than female rats to the antinociceptive action of morphine. The present study used age-matched (9–10 weeks old) male and female Sprague–Dawley rats to investigate whether this difference is due to variation in mu-opioid receptor binding and G protein activation. In the warm-water tail-withdrawal assay at both 50 °C and 55 °C, morphine was 2–3 times more potent in males than females. In contrast, mu-opioid receptor number and the binding affinity of the mu-opioid agonists morphine and DAMGO in membranes from whole brain, cortex, thalamus, and spinal cord were not different between males and females. Similarly, morphine and DAMGO stimulation of G protein, determined using GTPase and [$^{35}$S]GTPγS binding assays, did not show a difference between the sexes. The long-lasting mu-opioid receptor antagonist methocinnamox (0.32 mg/kg), given 24 h prior to morphine, reduced mu-opioid receptor number by approximately 50% in thalamic and spinal cord tissue from female and male rats and reduced the antinociceptive potency of morphine. Pretreatment of male rats with 0.32 mg/kg methocinnamox reduced the antinociceptive potency of morphine to that observed in female rats expressing a full complement of mu-opioid receptors. However, with increasing pretreatment doses of methocinnamox, the maximal antinociceptive effect of morphine was decreased in females but not males. The results suggest that pathways downstream of the mu-opioid receptor and G protein are more efficient in male rats than in female rats such that there is a larger receptor reserve for morphine-mediated antinociception.


*Theme:* Neurotransmitters, modulators, transporters, and receptors
*Topic:* Opioid receptors

*Keywords:* Mu-opioid receptor; G protein; Antinociception; Methocinnamox; Sex difference; Gender

## 1. Introduction

Male rodents are consistently more sensitive than female rodents to the acute antinociceptive effects of morphine in assays using a variety of nociceptive stimuli and behavioral end points [2–4,7–9,12,22–24,40,43,44]. This has also been reported in non-human primates [34]. The mechanism underlying this difference is not known, although the greater sensitivity of male rats to the antinociceptive actions of morphine only applies to acute administration since female rats have been reported to be more sensitive to chronic morphine due to greater tolerance development in male rats [39].

Morphine exerts its antinociceptive effect by interaction with mu-opioid receptors which are coupled to pertussis-toxin-sensitive G proteins. These G proteins activate several downstream effectors including inwardly rectifying potassium channels, voltage-gated calcium channels, and ade-

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nylyl cyclase. However, there is no difference in the binding of the mu-opioid ligand [$^3$H]DAMGO in hypothalamus and cortex between male and female Sprague–Dawley rats [25]. The affinity of morphine for these binding sites was not investigated, although in support of these data, no differences between male and female Lewis rats in the ability of the mu-agonists morphine, DAMGO, and buprenorphine to stimulate [$^{35}$S]GTPγS binding as a measure of G protein activation in thalamus, striatum, cingulate cortex, or periaquaductal gray was observed [38]. Moreover, no differences were seen in the ability of DAMGO to stimulate [$^{35}$S]GTPγS binding in the nucleus accumbens and striatum of Sprague–Dawley rats [30]. In contrast to these findings, one group using Swiss–Webster mice reported an increased level of mu-opioid binding in males compared to females with [$^3$H]naloxone [32], although another group reported no difference using [$^3$H]DAMGO [8]. Several studies do, however, point to differences in mu-opioid binding in sexually dimorphic hypothalamic regions of several species [21,28,36]. To complicate matters further, there is evidence in humans that females have a greater mu-opioid binding capacity in several cortical areas [48], yet in monkey cortical tissue, we have observed a higher number of mu-opioid binding sites in male monkeys than in female monkeys [26].

The differences in antinociceptive potency of morphine is conserved across several strains of rat, although the magnitude of the potency difference for morphine-mediated antinociception between female and male rats is not consistent across strains [14,33,43,44]. Consequently, the goal of the present study was to examine mu-opioid receptor number as well as morphine's affinity for mu-opioid receptors and its ability to activate G protein in age-matched (9–10 weeks old) male and female Sprague–Dawley rats obtained from the same supplier and housed under identical conditions in which a sex difference in morphine antinociception was observed. We and others [4,11–13,17,18,33] examining sex differences in morphine-mediated antinociception have used Sprague–Dawley rats. This strain shows an intermediate, but consistent, degree of sex difference in morphine-mediated antinociception with F344 and F344-Sasco strains showing a greater sex difference and the Long Evans–Blue Spruce, Holtzman, Brown Norway, and Long Evans strains showing a lesser degree of sex difference [43].

Antinociception was determined following s.c. morphine in the warm-water tail-withdrawal assay. Central nervous tissues were compared for mu-opioid receptor number and for morphine-stimulated G protein activation by [$^{35}$S]GTPγS binding and GTPase activity measures. In vitro assays were performed in spinal cord, thalamic, and cortical tissue. The warm-water tail-withdrawal test is an essentially spinally mediated response and we are not aware of any receptor or G protein studies of sex differences using spinal cord. In addition, thalamus and cortex are significantly involved in nociceptive and antinociceptive pathways [10] and in particular, the rat thalamus expresses a high density of mu-opioid receptors [29]. It has been reported that the irreversible mu-opioid antagonist β-funaltrexamine (β-FNA) has a more profound effect on the antinociceptive response to morphine in female rats than male rats. This indicates that there may be a greater mu-opioid receptor reserve and/or increased signaling efficiency in male rats compared to female rats [18]. However, the effect of β-FNA treatment on mu-opioid receptor number and in vitro efficacy was not assessed. Consequently, we have employed the more efficient and more selective long-lasting mu-opioid antagonist methocinnamox (MCAM) [7] to investigate a role for receptor reserve. Changes in the antinociceptive potency of morphine were measured along with mu-opioid receptor number and G protein activation.

The results confirm that morphine is more potent in male Sprague–Dawley rats than their female counterparts and that this is not due to a difference in their number of mu-opioid receptors or binding affinity or the efficiency and level of G protein coupling. MCAM treatment reduced the antinociceptive potency of morphine in both female and male rats. However, male rats required 50% fewer receptors to show the same antinociceptive response to morphine as naïve female rats, and with a high dose of MCAM (10 mg/kg), the morphine dose–response curve in females, but not males, was flattened. This indicates a more efficient system between G protein activation and antinociceptive response in the males.

## 2. Methods

### 2.1. Subjects

Female and male Sprague–Dawley rats (9–10 weeks of age, approximately 200 g and 300 g, respectively) were purchased from Harlan (Indianapolis, IN). For each warm-water tail-withdrawal assay, six male and six female rats were used. Rats were housed three to a cage with free access to standard laboratory diet chow and water. Animal quarters were maintained at 71–74 °F, on a 12:12-h light:dark cycle, with lights on at 6:30 am. Studies were performed in accordance with the Declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. Experimental animal protocols were approved by the University of Michigan University Committee on the Use and Care of Animals.

### 2.2. Chemicals and drugs

[$^{35}$S]GTPγS, [$^3$H]DAMGO ([D-Ala$^2$, Me-Phe$^4$,Gly(ol)$^5$] enkephalin; 42 Ci/mmol), and [$^3$H]diprenorphine (31 Ci/mmol) were from Perkin-Elmer Life Sciences (Boston, MA). Morphine, fentanyl, and naloxone were obtained through the Opioid Basic Research Center at the University of Michigan (Ann Arbor, MI). Methocinnamox (MCAM)