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Gender Determinants of Opioid Mediation of Swim Analgesia in Rats

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ROMERO, M.-T., K. L. KEPLER AND R. J. BODNAR. *Gender determinants of opioid mediation of swim analgesia in rats.* PHARMACOL BIOCHEM BEHAV **29**(4) 705–709, 1988.—Continuous cold-water swims (CCWS) and intermittent cold-water swims (ICWS) elicit respective nonopioid and opioid analgesic responses in adult male rats. The present experiment evaluated whether gender differences were observed in naloxone's (14 mg/kg, SC) ability to alter differentially CCWS and ICWS analgesia on the tail-flick and jump tests in age-matched and weight-matched intact rats and in gonadectomized rats. CCWS analgesia was unaffected by naloxone on either test in age-matched males and females. Naloxone significantly reduced ICWS analgesia on the tail-flick (45%) and jump (37%) tests in intact males, but not age-matched females. Naloxone significantly reversed ICWS analgesia in weight-matched males on the tail-flick (1–14 mg/kg, 30–32%) and jump (14 mg/kg, 31%) tests. Naloxone also significantly reduced ICWS analgesia on the tail-flick (32%) and jump (41%) tests in castrated males, but not ovariectomized females. Changes in swim hypothermia could not account for the above effects. These data indicate gender differences in naloxone's differential modulation of swim analgesia, and reflect further differences in pain-inhibitory responses as a function of gender.

Gender differences Naloxone Swim analgesia Gonadectomy Opiate/nonopiate analgesic responses Rats

IN the study of environmental activation of pain-inhibitory systems, parametric variables have played an important role in determining whether analgesia induced by cold-water swims is opioid-mediated or nonopioid-mediated [2, 3, 6–8]. In this regard, male rats exposed to continuous cold-water swims (CCWS) display an analgesia which is neither cross-tolerant with morphine analgesia nor significantly reduced by naloxone [2, 3, 5]. Indeed, CCWS analgesia is potentiated by the irreversible opiate receptor antagonist, naloxazone [10] and reduced by the putative anti-enkephalinase, D-phenylalanine [4]. In contrast, male rats exposed to intermittent cold-water swims (ICWS) display analgesia which is both cross-tolerant with morphine analgesia and significantly reduced by naltrexone [6–8]. Our laboratory has recently demonstrated that gender-specificity and gonadal function play important roles in determining the magnitude of both CCWS and ICWS analgesia. Female rats display significantly smaller analgesic responses following CCWS and ICWS than either age-matched or weight-matched male rats [11]. Further, gonadectomy reduces the magnitude of CCWS and ICWS analgesia for both genders [12]. The present study examined whether gender factors played a role in the opioid modulation of ICWS analgesia by evaluating whether naloxone altered CCWS and ICWS analgesia in female rats relative to age-matched and weight-matched male rats, and whether gonadectomy changed this relationship.

METHOD

Sprague-Dawley rats were housed in same-sex pairs in wire mesh cages, and were maintained on a 12 hr light:12 hr dark cycle at ambient temperatures between 21–25°C with rat chow and water available ad lib. All rats were tested for baseline tail-flick latencies and jump thresholds over four days as described previously (e.g., [11]). Seven male (430–550 g) and seven female (235–310 g) animals matched for age (99–100 days of age) were exposed in counterbalanced order to each of the following five conditions at weekly intervals: (a) no swim, (b) CCWS (2°C for 3.5 min), (c) CCWS 5 min following pretreatment with naloxone (14 mg/ml normal saline/kg body weight, SC), (d) ICWS (18 pairs of 10-sec swims at 2°C and 10-sec recovery periods), and (e) ICWS 5 min following pretreatment with naloxone (14 mg/kg, SC). To ascertain whether circulating gonadal steroids were responsible for any observed differences in naloxone effectiveness upon ICWS analgesia as a function of gender, ten male and ten female rats (90–100 days of age) were castrated and ovariectomized respectively as described previously [12]. After a 4-week period to allow development of gonadectomy-induced weight changes (−12 g gain in castrated rats, +30 g gain in ovariectomized rats relative to sham controls), each rat was exposed in counterbalanced order to the following three conditions at weekly intervals: (a) no swim, (b) ICWS and (c) ICWS 5 min following

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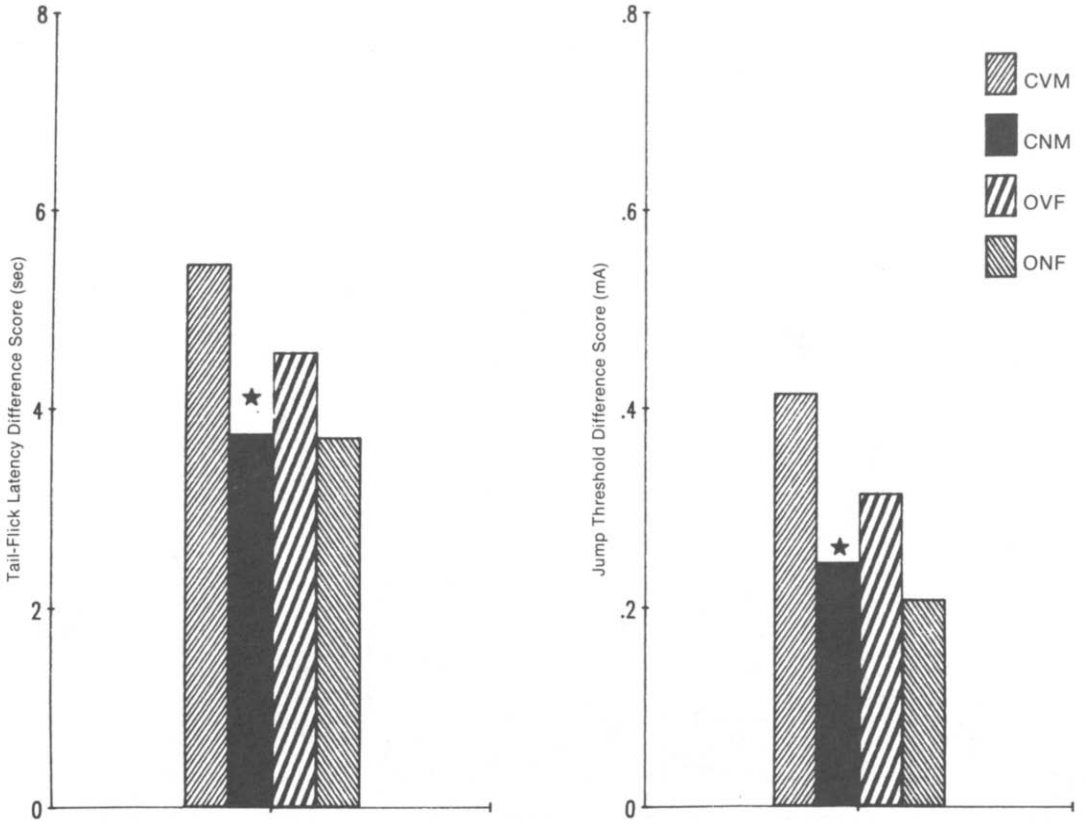

FIG. 1. Differential effectiveness of naloxone (14 mg/kg, SC) to alter intermittent cold-water swim (ICWS) and continuous cold-water swim (CCWS) analgesia on the tail-flick (left panel) and jump (right panel) tests in age-matched intact male (VM: vehicle males; NM: naloxone males) and intact female (VF: vehicle females; NF: naloxone females) rats 30 min following the swims. Significant differences in analgesia on the tail-flick test were observed between genders [CCWS: $F(1,12)=13.45$, $p<0.003$], between injection conditions [CCWS: $F(1,12)=8.19$, $p<0.014$], across the time course [ICWS: $F(3,36)=84.77$, $p<0.0001$; CCWS: $F=75.21$, $p<0.0001$] and for the interactions between injection condition and time [ICWS: $F(3,36)=5.98$, $p<0.002$] and among gender, injection condition and time [ICWS: $F(3,36)=3.97$, $p<0.015$]. Significant differences in analgesia on the jump test were observed between genders [ICWS: $F(1,12)=5.42$, $p<0.038$], between injection conditions [ICWS: $F(1,12)=21.47$, $p<0.0006$], across the time course [ICWS: $F(3,36)=125.20$, $p<0.0001$; CCWS: $F=35.07$, $p<0.0001$] and for all interaction conditions ($p<0.029$). Naloxone significantly reduced ICWS analgesia (dark stars: Dunnett comparisons, $p<0.05$) on the tail-flick and jump tests in intact males, but not age-matched intact females. Naloxone failed to significantly affect CCWS analgesia in either gender. As reported previously [11], gender differences in the magnitude of ICWS analgesia on the tail-flick test and CCWS analgesia on both tests was observed (enclosed stars: Dunnett comparison, $p<0.05$). Similar patterns of effects were observed at other post-swim intervals.

naloxone (14 mg/kg, SC). Tail-flick latencies, jump thresholds and rectal temperatures were assessed 30, 60, 90 and 120 min following each of the above conditions. The tester was unaware of the experimental status of each rat.

The antagonist dose was chosen because of its previously-established effectiveness in delineating opioid forms of footshock [9] and ICWS [7] analgesia in male rats; these studies also demonstrated antagonist effectiveness in males at lower doses with naltrexone. To assess whether naloxone possessed similar modes of action, a third group of ten male rats matched for weight (250–280 g, 60 days of age) with the females were exposed to the following five conditions in counterbalanced order at weekly intervals: (a) vehicle/no swim, and ICWS 5 min following either (b) vehicle, (c) 1 mg/kg, (d) 5 mg/kg and (e) 14 mg/kg of naloxone. Tail-flick latencies, jump thresholds and rectal temperatures were assessed 30, 60 and 90 min following each swim condition.

RESULTS

CCWS and ICWS Analgesia—Age-Matched Effects

Relative to the no-swim condition, tail-flick latencies and jump thresholds were significantly increased across the time course, and rectal temperatures were significantly decreased across the time course following all swim conditions in intact and gonadectomized rats (all main and interaction effects, $p<0.0001$). Since female rats displayed significantly higher baseline tail-flick latencies and significantly lower jump thresholds than male rats, and since changes in the magnitude of the analgesic effects were the focus of the study, analyses of variance were performed on difference scores of each post-swim value which was derived by subtracting each no-swim value from each corresponding post-swim score. The left panel of Fig. 1 illustrates the significant 45% decrease in ICWS analgesia on the tail-flick test in intact male

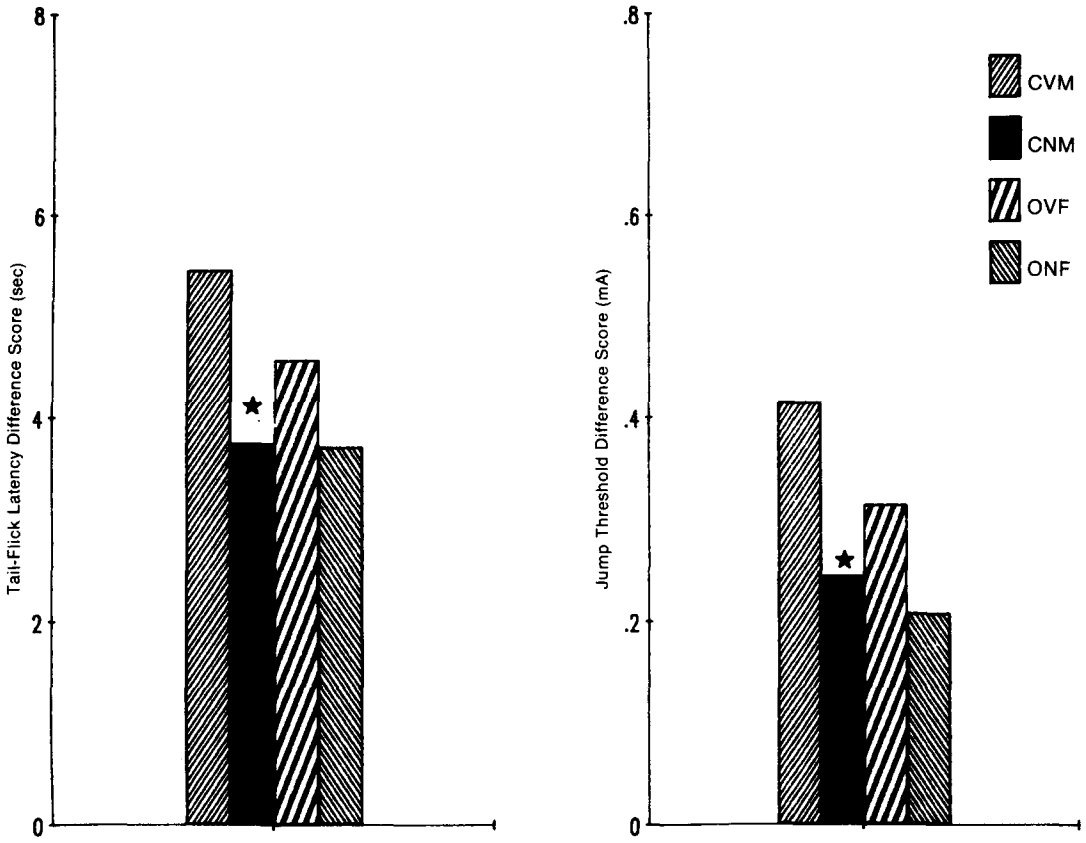

FIG. 2. Differential effectiveness of naloxone (14 mg/kg, SC) to alter ICWS analgesia on the tail-flick (left panel) and jump (right panel) tests in castrated male (CVM: castrated vehicle males; CNM: castrated naloxone males) and ovariectomized females (OVF: ovariectomized vehicle females; ONF: ovariectomized naloxone females) 30 min following the swims. Significant differences in ICWS analgesia were observed between injection conditions [tail-flick: $F(1,18)=5.73$, $p<0.028$; jump: $F=12.22$, $p<0.003$], across the time course [tail-flick: $F(3,54)=28.93$, $p<0.0001$; jump: $F=76.53$, $p<0.0001$] and for the interaction between injection condition and time [tail-flick: $F(3,54)=5.53$, $p<0.002$; jump: $F=8.32$, $p<0.0001$]. Naloxone significantly reduced ICWS analgesia (dark stars) on the tail-flick and jump tests in castrated males, but not ovariectomized females. Similar patterns of effects were observed at other post-swim intervals.

rats following the 14 mg/kg dose of naloxone. In contrast, the same naloxone dose failed to alter the magnitude (3% decrease) of ICWS analgesia on the tail-flick test in females. Naloxone failed to decrease significantly CCWS analgesia on the tail-flick test in either intact males (19% decrease) or intact females (24% decrease). The right panel of Fig. 1 illustrates this similar pattern of effects for the jump test: ICWS analgesia was significantly decreased by naloxone in intact male (37% decrease) but not intact female (9% decrease) rats. Again, CCWS analgesia on the jump test was not significantly altered by this dose of naloxone in either intact males (21% decrease) or intact females 11% decrease). The left panel of Fig. 2 illustrates the significant 32% decrease in ICWS analgesia on the tail-flick test in castrated males following naloxone. Naloxone failed to alter ICWS analgesia (19% decrease) on this measure in ovariectomized females. The right panel of Fig. 2 illustrates the significant 41% decrease in ICWS analgesia on the jump test in castrated males following naloxone. Naloxone failed to alter ICWS analgesia (32% decrease) on this measure in ovariectomized females. Gonadectomy was confirmed by a 92% reduction of prostate tissue in castrated males and a 77% reduction of uterine weight in ovariectomized females.

CCWS and ICWS Hypothermia—Age-Matched Effects

Significant differences in ICWS hypothermia were observed between genders, $F(1,12)=4.52$, $p<0.05$, among test times, $F(4,48)=347.40$, $p<0.0001$, and for the interactions between gender and time, $F(4,48)=6.64$, $p<0.0002$, and between injection condition and time, $F(4,48)=3.23$, $p<0.02$. Naloxone failed to affect ICWS hypothermia in intact and gonadectomized animals of either gender (data not shown). Significant differences in CCWS hypothermia were observed between injection conditions, $F(1,12)=4.97$, $p<0.046$, among test times, $F(4,48)=171.75$, $p<0.0001$, and for the interaction between gender and time, $F(4,48)=8.25$, $p<0.0001$. Naloxone failed to affect CCWS hypothermia in either intact males or females (data not shown).

ICWS Analgesia and Hypothermia—Weight-Matched Males

ICWS produced significant analgesia across the time

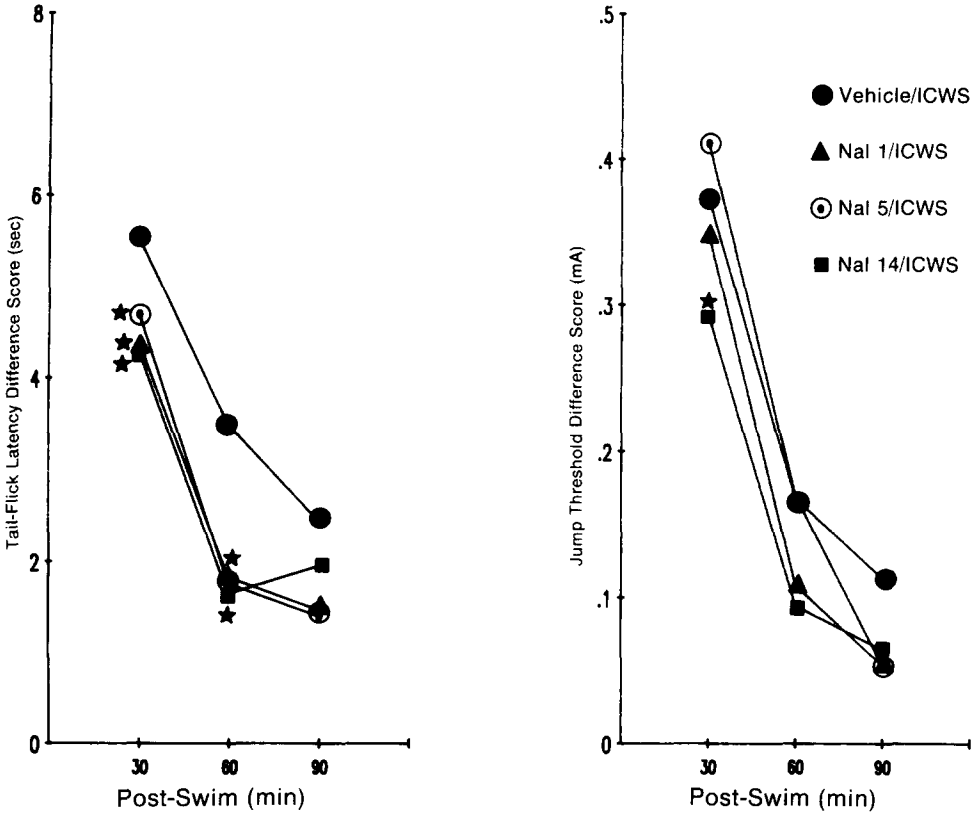

FIG. 3. Alterations in ICWS analgesia on the tail-flick (left panel) and jump (right panel) tests following pretreatment with either vehicle or naloxone (Nal) at doses of 1, 5, or 14 mg/kg administered subcutaneously to male rats matched for weight with female rats described in Fig. 1. Significant differences in ICWS analgesia were observed among injection conditions [tail-flick: $F(3,27)=3.09$, $p<0.044$; jump: $F=2.57$, $p<0.092$, trend] and among test times [tail-flick: $F(2,18)=15.50$, $p<0.0001$; jump: $F=47.10$, $p<0.0001$]. All doses of naloxone significantly reduced (dark stars: Dunnett comparisons, $p<0.05$) ICWS analgesia on the tail-flick test relative to vehicle pretreatment. The 14 mg/kg, but not the 1 or 5 mg/kg doses of naloxone significantly reduced ICWS analgesia on the jump test.

course in all swim groups on the tail-flick ($p<0.01$) and jump ($p<0.0001$) tests. The left panel of Fig. 3 illustrates the significant reduction in ICWS analgesia on the tail-flick test in young male rats following the 1 (30 and 60 min, 32% overall decrease), 5 (60 min, 31% overall decrease) and 14 (30 and 60 min, 30% overall decrease) mg/kg doses of naloxone. The right panel of Fig. 3 illustrates the significant reduction in ICWS analgesia on the jump test in young male rats following the 14 mg/kg (30 min, 31% overall decrease), but not the 1 or 5 mg/kg doses of naloxone. Significant differences in ICWS hypothermia were observed among the experimental treatments, $F(4,36)=19.72$, $p<0.0001$, among test times, $F(2,18)=854.60$, $p<0.0001$, and for the interaction between treatments and times, $F(8,72)=19.21$, $p<0.0001$. ICWS produced significant hypothermia across the time course in all swim groups. However, none of the naloxone doses altered ICWS hypothermia (data not shown).

DISCUSSION

The present study confirmed that gender alters the magnitudes of CCWS and ICWS analgesia: females display a smaller effect than male rats [11]. The present study also revealed that gender affects the ability of the opiate receptor antagonist naloxone to attenuate differentially the analgesic response following CCWS and ICWS. Naloxone (14 mg/kg) significantly reduced ICWS analgesia on the tail-flick and jump tests in mature (90–100 days of age) intact male rats, confirming the initial finding of Girardot and Holloway [7]. The same dose of naloxone failed to attenuate significantly CCWS analgesia in these intact male rats, confirming initial findings from our laboratory [2,5]. Indeed, these data are consistent with previous findings in that: (a) ICWS analgesia was not completely reversed by naloxone, but rather significantly attenuated [7]; and (b) the (opioid-nonopioid) dichotomy of ICWS and CCWS analgesia respectively is a matter of degree of opiate antagonist effectiveness, and not a matter of total presence or absence of an effect [2, 5, 7]. This latter point is substantiated by the ability of naloxone in intact males to reduce significantly ICWS analgesia (tail-flick: 45%, jump: 37%), yet nonsignificantly reduce CCWS analgesia (tail-flick: 19%, jump: 21%). While naloxone was capable of differentiating these two analgesic responses in intact males, it failed to do so in age-matched intact females. As expected of an essentially nonopioid response [2–5, 10], naloxone failed to significantly affect CCWS analgesia (tail-flick: 24%, jump: 11%) in intact females. However, while ICWS analgesia is opioid-mediated in male rats [6–8], naloxone failed to significantly affect ICWS analgesia (tail-flick: 3%, jump: 9%) in intact females, suggesting that ICWS analgesia in intact females is essentially nonopioid.

However, before such a conclusion was warranted, several aspects of the above findings needed corroboration. First, since age-matched males and females differed in body weight by 240 g and since naloxone was administered on a mg/kg basis, the effect could be due to a larger amount of naloxone reaching relevant target structures in males. Second, we observed differential effects upon ICWS analgesia with naloxone, although the original opioid delineation of ICWS analgesia was determined with naltrexone, a longer-acting antagonist [7]. The 14 mg/kg dose was chosen because of its previous use in both footshock and ICWS analgesia paradigms [7,9]; however, this dose raises questions of specificity upon opioid systems (see reviews: [13,14]). Therefore, the dose-dependent effects of naloxone upon ICWS analgesia were evaluated in a sexually-mature (60 days of age) group of male rats that were matched for body weight relative to females. The significant reduction of ICWS analgesia by naloxone in this group indicated that gender, but not weight, differences were responsible for the differential naloxone effect on ICWS analgesia. Naloxone was more sensitive in reducing ICWS analgesia on the tail-flick (1–14 mg/kg, 30–32%) than on the jump (14 mg/kg, 31%) test. It is conceivable that the failure of naloxone to alter ICWS analgesia in intact females was due to uncontrolled estrous influences since systemic morphine analgesia is sensitive to estrous changes [1]. We [11] previously observed that CCWS analgesia is not subject to estrous influences, but have not tested ICWS analgesia on this measure. However, if circulating gonadal steroids were responsible for this differential gender-specific naloxone effect, then elimination of these steroids by gonadectomy should cancel the differential effect. It does not; castrated males, like their intact male counterparts display ICWS analgesia which is significantly reversed by naloxone. Ovariectomized females, like their intact female counterparts fail to display significant naloxone reversibility. Further studies must be conducted to definitively assess estrous influences. Finally, the failure of naloxone to significantly affect ICWS hypothermia suggests that the differential gender effects of naloxone upon ICWS analgesia in intact and gonadectomized rats was probably due to differential mediation of intrinsic pain-inhibitory systems.

ACKNOWLEDGEMENTS

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