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Roles of Gender, Gonadectomy and Estrous Phase in the Analgesic Effects of Intracerebroventricular Morphine in Rats

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KEPLER, K L , B KEST, J M KIEFEL, M L COOPER AND R J BODNAR *Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats* PHARMACOL BIOCHEM BEHAV **34**(1) 119–127, 1989 —Gender and gonadal function have previously been shown to influence the magnitude of analgesia following systemic morphine and opioid and nonopioid forms of swim analgesia with male rats displaying greater analgesia than female rats and gonadectomy reducing analgesic magnitude in both genders These effects have been presumed to be centrally mediated The present study evaluated the roles of gender, gonadectomy and estrous phase upon dose-response and time-response functions of analgesia following intracerebroventricular administration of morphine as measured by the tail-flick and jump tests Sham-operated male rats displayed significantly greater magnitudes of peak and total analgesia following central morphine than sham-operated female rats on both nociceptive measures This striking effect was reflected both in terms of magnitude and ED_{50}, while male rats displayed near-maximal analgesia at a 5 μg dose of morphine, female rats displayed moderate analgesia at doses as high as 40 μg of morphine Castration produced small, but significant reductions in the magnitude of central morphine analgesia, the ED_{50} of morphine analgesia, however, was not changed Although female rats in either proestrous or estrous displayed significantly greater magnitudes of analgesia than ovariectomized rats or rats in a combined met-/di-estrous phase at some doses, the ED_{50} of morphine analgesia was not significantly altered as functions of estrous phase or ovariectomy The interaction of opiate receptors and gonadal steroid receptors is considered as one possible determinant of gender differences observed in the magnitude and potency of central morphine analgesia

Central morphine analgesia Gender differences Gonadectomy Estrous phases Pain Rats

THE roles of gender and gonadal function in the mediation of opioid and nonopioid forms of pain inhibition have been recently elucidated [see review (7)]. Female rats display significantly lower shock thresholds than male rats (4,37) While androgenized female rats display shock thresholds similar to intact male rats, castrated male rats display shock thresholds similar to intact female rats (5). Female rats exhibit their greatest basal sensitivity to shock during the estrous phase (12). The analgesic magnitudes of systemic morphine, opioid intermittent cold-water swims (ICWS) (6) and nonopioid continuous cold-water swims (CCWS) (6) are higher in intact male than female rats (2, 23, 40) Gonadectomy reduces each of these forms of analgesia relative to same sex controls (9,42), and steroid replacement therapy with testosterone reverses the swim analgesia deficits in gonadectomized animals (41) While the phase of estrous fails to alter nonopioid CCWS analgesia (40), the greatest sensitivity to systemic morphine analgesia occurs during diestrous in intact female rats (3).

The site of action for these gender and gonadectomy effects upon analgesic processes has been presumed to be centrally mediated. The present study tested this hypothesis by evaluating the relative importance of three gender-related variables, gender differences, gonadectomy and estrous phase, upon the central analgesic effects of morphine following intracerebroventricular administration [see review, (51)] Dose-response and time-response actions of central morphine analgesia were evaluated in sham-operated male rats, castrated male rats, ovariectomized female rats, and sham-operated female rats during estrous, pro-

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estrus, and met-/di-estrous on the spinally mediated tail flick (11) and the supraspinally mediated jump (13) tests

METHOD

Subjects and Surgery

One hundred and twenty albino Sprague-Dawley rats (90–100 days of age, Charles River Laboratories) were housed individually in flat-bottomed plastic cages in the Queens College vivarium and were maintained on a 12-hour light (0800 hr) 12 hour dark (2000 hr) cycle with Purina rat chow and water available ad lib Male (approximately 390–475 g) and female (approximately 290–325 g) rats were matched into sham and gonadectomy groups on the basis of preoperative body weights All rats were anesthetized with Ketamine (100 mg/ml sterile water/kg body weight, IM) Castrations were performed by removing the testes and testicular fat following a single 2 cm midscrotal incision (42) Ovariectomies were performed by removing the ovaries and ovarian fat through a dorsal approach (42) Sham surgeries for each gender occurred in which the organs were exposed, but not removed All rats were weighed at least one month after gonadal surgery to assess any gonadectomy-induced weight changes (6) At this time, all rats were pretreated with chlorpromazine (3 mg/kg, IP) and anesthetized with Ketamine (100 mg/kg, IM) A stainless steel guide cannula (22 gauge, Plastic Products) was placed stereotaxically (Kopf Instruments) 0 3 mm above the left lateral ventricle by using the following coordinates incisor bar (+5 mm), 0 5 mm anterior to the bregma suture, 1 3 mm lateral to the sagittal suture and 3 6 mm from the top of the skull The cannula was secured to the skull by anchor screws with dental acrylic All animals were allowed at least one week to recover from stereotaxic surgery before behavioral testing began

Gonadal and Histological Analysis

After the last experimental session, each animal received an overdose of anesthesia (Euthanasia, No 5, H Schein and Co) With the bladder and urethral region exposed, the seminal vesicles were dissected in the males, blotted dry, and organ weights determined (42) The fallopian tubes of females were likewise dissected, dried and weighed (42) As the animals could not be perfused due to analysis of gonadal tissue, the unfixed brains were removed, blocked and were coronally sectioned through the lateral ventricle to determine cannula placement Any animal with a cannula placement that missed the lateral ventricle was excluded from data analysis

Nociceptive Tests

All rats were tested on the tail-flick and jump tests The stimulus source (IITC, Woodland Hills, CA) was mounted 8 cm above the dorsum and 3–8 cm proximal to the tip of the tail of a lightly restrained animal The intensity of the thermal stimulus was set to produce stable baseline tail-flick latencies between 2 5 and 4 sec Each tail-flick test session consisted of three latency determinations In order to avoid tissue damage, a trial was automatically terminated if a response did not occur within 15 sec Immediately following latency determinations, the rats were placed in a 30 cm by a 24 cm chamber with a floor consisting of 16 grids Electric shock was delivered to the grids by a shock generator (BRE/LVE) through a shock scrambler (Campden Instruments) Using an ascending method of limits procedure, the jump threshold was defined in mA as the lower of two consecutive intensities in which the animal simultaneously removed both hindpaws from the grids or when the intensity reached 1 0 mA to avoid tissue damage Each trial began with the animal receiving a 300-msec footshock at a current intensity of 0 10 mA Subsequent shocks were increased in 0 05-mA increments at 10 sec intervals until the jump threshold was determined After each trial, the current intensity was reset to 0 10 mA and the procedure was repeated until six trials were completed The order of tail-flick latency and jump threshold determinations was employed because it yields minimal carry-over effects in baseline testing within a test period (24)

Protocol

All intracerebroventricular injections were made in a 5-μl volume of normal saline and infused (Hamilton syringe and polyethylene tubing) at a rate of 1 μl every 15 sec through a stainless steel internal cannula (28 gauge, Plastic Products) which protruded 0 5 mm beyond the tip of the guide cannula into the lateral ventricle All testing took place between 2 and 10 hr into the light cycle to control for basal and opiate circadian oscillations (15,23) with resultant limitations on interpretation All rats received a maximum of three injection conditions, including a vehicle injection, treatment conditions were separated by at least one week to minimize possible tolerance effects (52) All rats were tested at 30, 60, 90, and 120 min after each microinjection on the tail-flick and jump tests, this interval between tests yields stable baseline and vehicle data across the time course (2, 40–42) Morphine doses of 1, 5, 10, 20 and 40 μg, dissolved in normal saline, were employed to construct dose-response and time-response curves, doses over 40 μg were not used because of their possible seizure effects (47) Estrous phase was monitored in sham-operated female rats using daily vaginal smears to determine the proestrus, estrus, or combined met-/di-estrus phases of the cycle, females were tested only within one phase of the estrous cycle Vaginal smears were taken 0–1 hr into the light cycle and experimental testing occurred between 1 and 7 hr after smears It should be noted that vaginal smears were taken on successive days before the experimental procedure began to adapt animals to the potential stressful consequences of the procedure Although vaginal probing produces analgesia (10,26), its time course of action completely dissipates within 2 min and the applied force necessary to produce analgesia far exceeds the smear procedure

Statistical Analyses

Two statistical approaches were utilized to analyze the data in terms of magnitude and potency (ED_{50}) of effect The magnitude of effect was evaluated using split-plot analyses of variance corrected for repeated measures on each dependent variable (tail-flick latencies and jump thresholds) to assess significant differences between vehicle and each individual morphine dose (1, 5, 10, 20, 40 μg) treatment among groups (sham males, sham females in each estrous phase, and gonadectomized males and females) and across test times (30–120 min) Individual determination of significant drug effects from corresponding vehicle control conditions were assessed with Dunnett comparisons ($p<0$ 05) Analysis of significant drug effects across groups at corresponding times and doses was assessed using difference scores which were derived by subtracting each postdrug effect from its corresponding vehicle value, Dunn comparisons ($p<0$ 05) were then used to evaluate differences among groups The potency of effects was evaluated by constructing log dose response functions by performing a linear regression analysis and indicating potency as the ED_{50} for peak and total analgesic effects for each nociceptive measure The criterion used for the ED_{50} was that minimal morphine dose which elicited a 50% increase relative to vehicle values for peak or total effects Calculations from the

TABLE 1

ALTERATIONS IN BODY WEIGHTS AND ACCESSORY SEXUAL ORGAN WEIGHTS FOLLOWING SHAM OR GONADAL SURGERY

Group	Preoperative Body Weight (g,SEM)	Postoperative Body Weight (g,SEM)	Accessory Sexual Organ Weight (mg,SEM)
Males (n)			
Sham (8)	409 (20)	483 (30)	940 (81 8)
Castrated (11)	455 (27)	523 (18)	152 (10 9)
% Change	—	−4%	−84%*
Females (n)			
Sham (33)	315 (9)	344 (10)	734 (38 5)
Ovariectomized (9)	309 (14)	362 (13)	248 (31 5)
% Change	—	+8%*	−67%*

Note The asterisks denote significant differences between the sham and gonadectomy conditions ($p<0$ 05). Percent weight gains were calculated for each group, the % change represents the difference between the sham and gonadectomy conditions The accessory sexual organs measured were the seminal vesicles in males and the fallopian tubes in females

TABLE 2

BASAL TAIL-FLICK LATENCIES AND JUMP THRESHOLDS (MEAN, SEM) FOLLOWING SHAM OR GONADAL SURGERY

Group	Tail-Flick Latencies (sec)	Jump Thresholds (mA)
Males (n)		
Sham (11)	3 25 (0 32)	0 460 (0 012)
Castrated (14)	3 03 (0 24)	0 435 (0 009)
Females (n)		
Proestrus (9)	3 46 (0 28)*	0 448 (0 016)
Estrous (11)	2 73 (0 12)	0 431 (0 014)
Met-/Di-estrous (11)	2 72 (0 19)	0 430 (0 012)
Ovariectomy (13)	3 74 (0 27)*	0 458 (0 018)

Note The asterisks denote significant differences in tail-flick latencies in female rats relative to the other phases of the estrous cycle

linear regression analyses allowed for the determination of significant differences between slopes and intercepts across groups by evaluating confidence intervals (95%)

RESULTS

Body Weight and Accessory Sexual Organs

Significant differences in body weight were observed among sham and gonadectomized males and females, $F(3,57) = 29\ 76$, $p<0\ 0001$, between pre- and postoperative measures, $F(1,57) = 55\ 33$, $p<0.0001$, and for the interaction between groups and times, $F(3,57) = 2.86$, $p<0.045$. Table 1 indicates that ovariectomized and sham-operated female rats gained 53 g (17% increase) and 29 g (9% increase) respectively Thus, gonadectomy in females significantly accelerated weight gain in female rats Castrated and sham-operated male rats gained 68 g (14% increase) and 72 g (18% increase) respectively, these effects failed to achieve significance Significant differences in the weights of both seminal vesicles of male rats, $F(1,17) = 125\ 74$, $p<0\ 0001$, and fallopian tubes of female rats, $F(1,42) = 49\ 44$, $p<0\ 0001$, were observed between sham-operated and gonadectomized animals of each gender, such that castration and ovariectomy significantly reduced the weights of these accessory sexual organs by 84% and 67% respectively (Table 1)

Baseline Nociceptive Thresholds

Table 2 indicates the significant differences in baseline tail-flick latencies among groups, $F(5,64) = 4\ 27$, $p<0\ 002$ Although sham-operated and castrated males failed to show basal differences in tail-flick latencies, ovariectomized females and sham-operated females in the proestrus phase displayed significantly shorter tail-flick latencies than sham-operated females in estrous or in the combined met-/di-estrous phases. In contrast, significant differences in baseline jump thresholds failed to occur among groups, $F(5,63) = 1\ 06$

Overall Analgesic Effects

Significant differences were observed for the following doses a) 1 μg groups [tail-flick $F(5,25) = 4\ 68$, $p<0\ 004$, jump $F = 4\ 79$, $p<0\ 003$], treatment [tail-flick $F(1,25) = 7\ 56$, $p<0\ 011$, jump $F = 9.49$, $p<0\ 005$], test times (jump $F = 5\ 49$, $p<0\ 002$), b) 5 μg groups [tail-flick $F(5,33) = 2\ 91$, $p<0.028$, jump $F = 5\ 93$, $p<0\ 001$], treatment [tail-flick $F(1,33) = 13\ 48$, $p<0\ 001$, jump $F = 60\ 28$, $p<0\ 001$], test times (jump $F = 11\ 53$, $p<0.001$), c) 10 μg groups [tail-flick $F(5,32) = 4\ 55$, $p<0\ 003$, jump $F = 4\ 35$, $p<0.004$], treatment [tail-flick $F(1,32) = 6\ 89$, $p<0\ 013$, jump $F = 72\ 29$, $p<0.001$], test times [tail-flick $F(3,96) = 6\ 05$, $p<0\ 001$, jump $F = 11\ 13$, $p<0\ 001$], d) 20 μg groups [tail-flick $F(3,21) = 3\ 24$, $p<0\ 043$], treatment (jump $F = 29\ 51$, $p<0\ 001$), test times (jump $F = 22\ 58$, $p<0\ 001$), e) 40 μg treatment [tail-flick $F(1,28) = 7\ 23$, $p<0\ 012$, jump $F = 49\ 38$, $p<0\ 001$], test times [tail-flick $F(3,84) = 6\ 34$, $p<0\ 001$, jump $F = 10\ 36$, $p<0\ 001$]

Central Morphine Analgesia Estrous and Gonadal Status in Female Rats

Figures 1 and 2 respectively display the differences in the magnitude of peak (60 min) and total (2-hr time course) morphine analgesia on the tail-flick (left panels) and jump (right panels) tests following central injections in ovariectomized rats and rats tested in the proestrus, estrous and combined met-/di-estrous phases Tables 3 and 4 summarize the ED_{50} potency effects of peak and total morphine analgesia on the tail-flick and jump tests respectively Although small, significant changes in central morphine analgesia occurred on both nociceptive measures as functions of estrous phase and female gonadectomy (Figs. 1 and 2), the regression analyses indicated that neither estrous phase nor ovariectomy significantly altered the slope or intercepts of the log dose response functions (Tables 3 and 4) Indeed, only rats tested in the proestrous phase displayed supracriterion values on the tail-flick and jump tests in which changes in analgesic magnitude exceeded 50% over baseline values. The following significant increases were observed a) ovariectomy (tail-flick peak, 1 and 40 μg, 2–45%, total, 1 μg, 3–36%, jump peak, 1–40 μg, 14–39%, total, 1–40 μg, 12–30%), b) met-/di-estrous (tail-flick peak, 1–40 μg, 7–47%, total, 1–40 μg, 5–38%, jump peak, 1–40 μg, 16–49%, total, 1–40 μg, 15–39%), c) proestrous (tail-flick peak, 1–40 μg, 17–139%, total, 1–40 μg, 3–123%, jump peak, 1–40 μg, 12–83%, total, 1–40 μg, 10–66%), d) estrous (tail-flick peak, 1–40 μg, 1–50%, total, 1–40 μg, 1–64%, jump peak, 1–40 μg, 8–67%, total, 1–40 μg, 4–57%) At the most effective dose of morphine in female rats (40 μg), a rank-order of peak and total

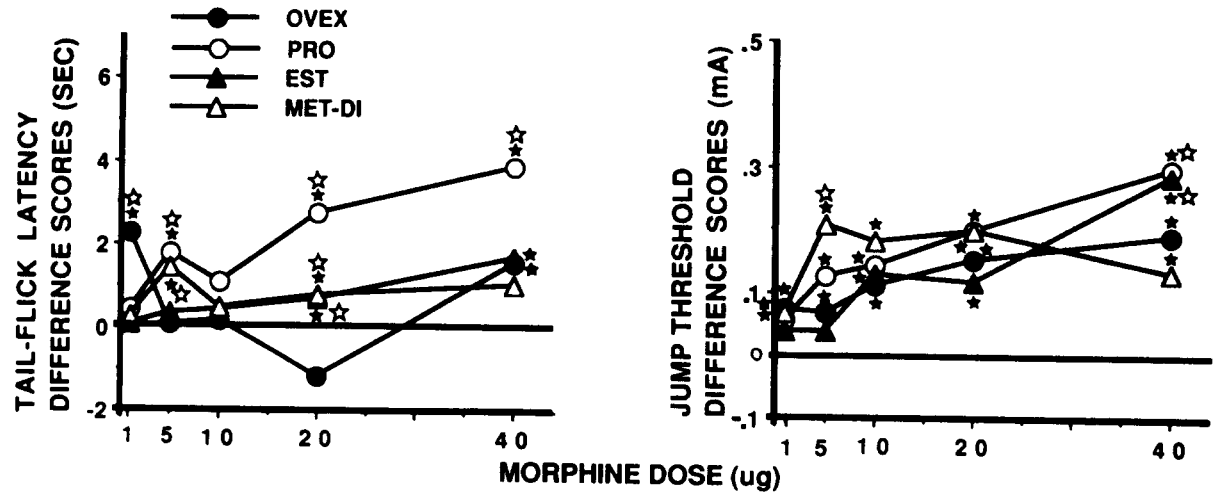

FIG 1 Alterations in peak analgesia 60 min following intracerebroventricular administration of morphine on the tail-flick (left panel) and jump (right panel) tests as functions of estrous phase and gonadectomy in female rats In this and subsequent figures, the data are expressed as difference scores which were derived from subtracting each experimental score from its corresponding vehicle control score The closed stars denote significant differences between the experimental and control values (Dunnett comparisons, $p<0$ 05) The open stars denote significant differences among the different estrous phases relative to ovariectomized animals (Dunn comparisons, $p<0$ 05) Separate groups of animals were tested at each dose point for each group ovariectomy (n = 5–11 rats), proestrus (n = 3–8 rats), estrous (n = 4–9 rats), met-/di-estrous (n = 3–6 rats)

analgesic effects across tests was proestrous > estrous > ovariectomy = met-/di-estrous To assess gender effects in the next section, the estrous phases were combined in sham-operated females

Central Morphine Analgesia Gender and Gonadectomy Effects

Figures 3 and 4 display respectively the peak (60 min) and total analgesic effects of morphine on the tail-flick (left panels) and jump (right panels) tests following intracerebroventricular administration in sham-operated and gonadectomized male and female rats Significant gender effects were observed for central morphine analgesia both in terms of magnitude of effects (Figs 3 and 4) and effective doses (Tables 3 and 4) for both nociceptive tests At a dose of 5 μg, the magnitude of morphine analgesia observed in sham-operated male rats relative to sham-operated female rats was five and three times greater for peak analgesia on the tail-flick and jump tests respectively Similarly, the magnitude of morphine (5 μg) analgesia observed in sham-operated male rats relative to sham-operated female rats was seven and three times greater for total analgesia on the tail-flick and jump tests respectively. In short, latencies and thresholds of male sham-operated rats were

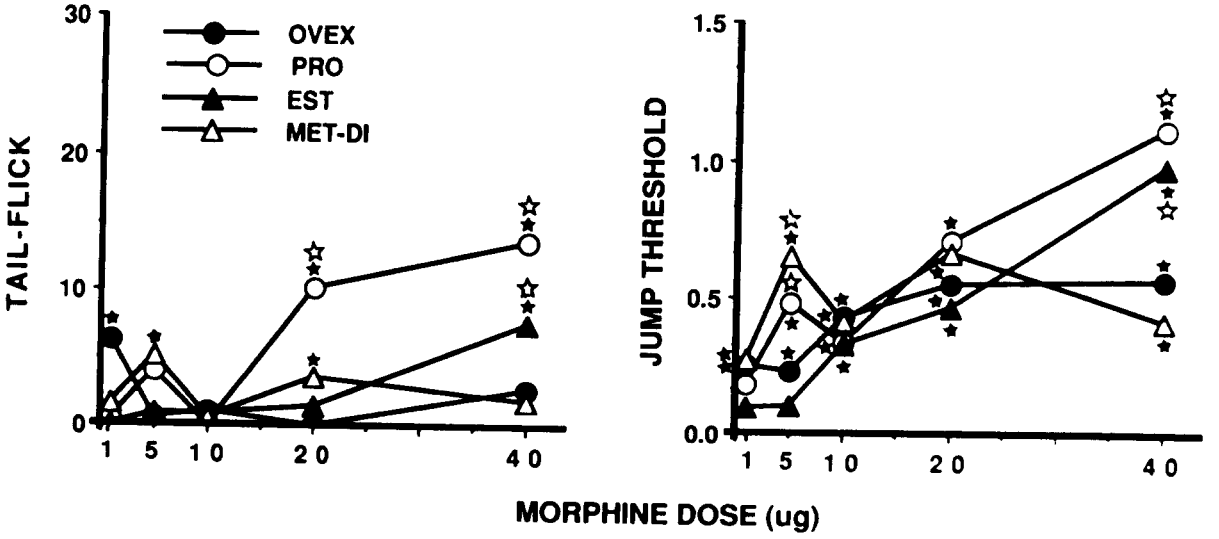

FIG 2 Alterations in total analgesia following intracerebroventricular administration of morphine on the tail-flick and jump tests as functions of estrous phase and gonadectomy in female rats Total analgesia was defined as the sum of the difference scores derived from the 30, 60, 90 and 120 min experimental time course The closed stars denote significant differences between total experimental and control values (Dunnett comparisons, $p<0$ 05) The open stars denote significant differences among the different estrous phases relative to ovariectomized animals (Dunn comparisons, $p<0$ 05)

TABLE 3

REGRESSION ANALYSIS OF THE LOG DOSE/RESPONSE FUNCTIONS OF CENTRAL MORPHINE ANALGESIA IN SHAM AND GONADECTOMIZED MALE AND FEMALE RATS ON THE TAIL-FLICK TEST

Group	ED_{50}* (μg)	Slope	Intercept	Standard Error of Estimate
		A Peak Tail-Flick Latencies		
Males (n)				
Sham (11)	1 0	7 96	1 47	4 35
Castrated (14)	1 0	4 13	2 06	5 04
Females (n)				
Proestrus (9)	2 5	1 93	0 46	3 97
Estrous (11)	40 0	1 06†	0 31	1 66
Met-/Di-estrous (11)	40 0	0 51†	0 24	1 01
Ovariectomy (13)	40 0	−0 74†	1 39	1 69
		B Total Tail-Flick Latencies		
Males (n)				
Sham (11)	1 7	33 62	2 94	16 95
Castrated (14)	1 0	8 12	7 47	16 97
Females (n)				
Proestrus (9)	10 5	7 61	−1 05	13 17
Estrous (11)	40 0	3 99†	−1 53	8 12
Met-/Di-estrous (11)	40 0	0 64†	1 97	2 94
Ovariectomy (13)	40 0	−2 52†	4 51	3 79

*The ED_{50} is defined that minimal morphine dose which elicits a 50% increase in baseline latencies for peak (60 min) effects or for total (the 120 min time course) effects

†Significant difference relative to sham males (confidence intervals 95%)

TABLE 4

REGRESSION ANALYSIS OF THE LOG DOSE/RESPONSE FUNCTIONS OF CENTRAL MORPHINE ANALGESIA IN SHAM AND GONADECTOMIZED MALE AND FEMALE RATS ON THE JUMP TEST

Group	ED_{50}* (μg)	Slope	Intercept	Standard Error of Estimate
		A Peak Jump Thresholds		
Males (n)				
Sham (11)	2 1	0 536	−0 031	0 140
Castrated (14)	1 9	0 300	0 074	0 162
Females (n)				
Proestrus (9)	15 1	0 155†	0 022	0 156
Estrous (11)	25 1	0.146†	−0 008	0 126
Met-/Di-estrous (11)	40 0	0 043†	0 113	0 129
Ovariectomy (13)	40 0	0.083†	0 043	0 102
		B Total Jump Thresholds		
Males (n)				
Sham (11)	1 8	1 936	0 026	0 472
Castrated (14)	2 6	1 015	0 193	0 593
Females (n)				
Proestrus (9)	18 6	0 505†	0 141	0 610
Estrous (11)	40 0	0 541†	−0 098	0 401
Met-/Di-estrous (11)	40 0	0 186†	0 234	0 010
Ovariectomy (13)	40 0	0 243†	0 191	0 311

*The ED_{50} is defined that minimal morphine dose which elicits a 50% increase in baseline thresholds for peak (60 min) effects or for total (the 120 min time course) effects

†Significant difference relative to sham males (confidence intervals 95%)

either at or close to cut-off values following the 5 μg dose of morphine Indeed, the gender differences were still apparent if the comparisons of analgesic magnitude involved the 5 μg dose for sham-operated males and the 40 μg dose for sham-operated females. peak analgesia (tail-flick test 300%; jump test· 38%) and total analgesia (tail-flick test 300%; jump test. 64%). Linear regression analyses revealed significant shifts in the slope, but not in the intercepts of the log-dose response functions of sham-operated male rats relative to all female groups on the jump test (Table 4), and to all but the proestrous group on the tail-flick test (Table 3) Relative to sham-operated males, shifts in the ED_{50} of peak and total morphine analgesia on both nociceptive tests were 20–40-fold for ovariectomized and met-/di-estrous female rats, 12–40-fold for estrous female rats and 2.5–20-fold for proestrous female rats. In contrast, the effects of male gonadectomy were not as striking Male sham-operated and castrated rats failed to exhibit differences in the ED_{50} of peak or total morphine analgesia on either measure (Tables 3 and 4) despite significant differences in analgesic magnitude following the 1 μg dose of morphine for peak analgesia on the tail-flick test, and following the 5 μg dose of morphine for total analgesia on the jump test. Comparable ranges (1–5 μg) of analgesic magnitude were observed for peak analgesia on the tail-flick (shams 38–188%, castrates: 6–183%) and the jump (shams 7–72%, castrates. 2–74%) tests and for total analgesia on the tail-flick (shams 19–190%; castrates· 0–140%) and the jump (shams 0–70%, castrates 0–56%) tests. Thus, a rank-order potency of analgesic magnitude was sham-operated males ≥ castrated males >> sham-operated females ≥ ovariectomized females

DISCUSSION

The present study evaluated whether central morphine analgesia following intracerebroventricular administration was altered by gender differences, gonadectomy and estrous phase. The most striking effects in the present data were gender effects sham-operated male rats displayed more marked analgesic magnitude and potency following central morphine on both nociceptive measures for both peak and total analgesic effects than sham-operated female rats. A 5 μg dose of morphine produced near-total analgesia in sham-operated males with significant numbers of the animals approaching or reaching cut-off values; this dose range is consistent with effects previously observed [see review, (51)] Comparisons of these effects with effects induced in sham-operated females at either an identical or maximal (40 μg) dose revealed a similar pattern of maximal effects in males. Thus, the gender differences in central morphine analgesia are similar in pattern to previously reported effects following peripheral administration of morphine (2,23), and suggest that the latter effects were not due to such pharmacokinetic factors as differences in drug absorbance, drug storage, and drug release The gender differences observed for central morphine analgesia are also similar in pattern to those observed for opioid and nonopioid forms of swim analgesia (40)

Although significant individual changes in the magnitude of central morphine analgesia were observed as functions of gonadectomy and estrous phase, regression analyses revealed that castrated and sham-operated male rats failed to differ significantly from each other in analgesic potency This effect stands in contrast

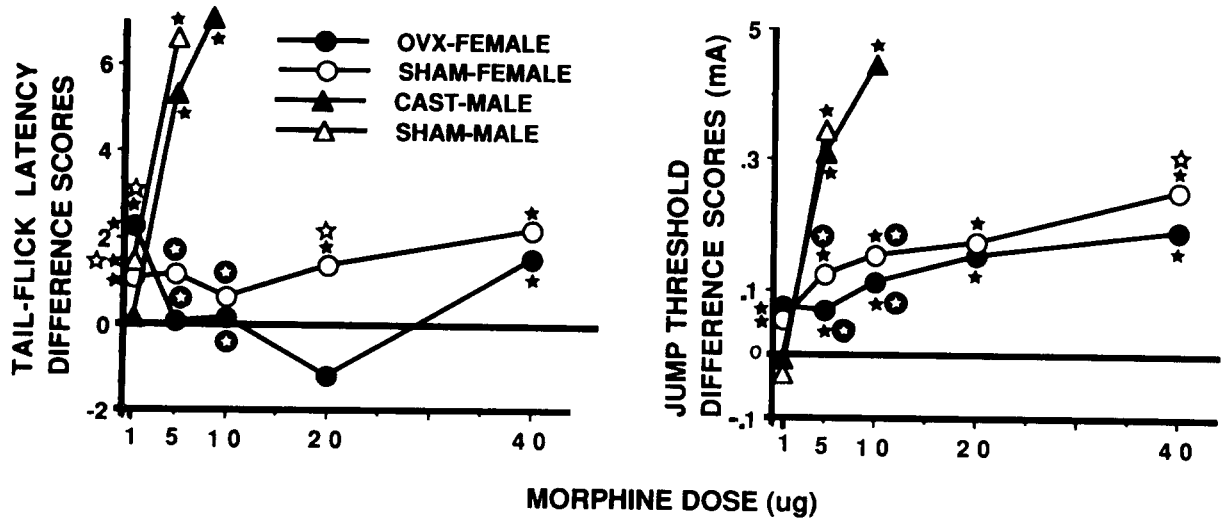

FIG 3 Alterations in peak analgesia 60 min following intracerebroventricular administration of morphine on the tail-flick and jump tests as functions of gender and gonadectomy The peak data of sham-operated females constituted the mean values of the proestrus, estrous and combined met-/di-estrous groups The closed stars denote significant differences between the experimental and control values (Dunnett comparisons, $p<0$ 05) The open stars and enclosed stars denote significant gonadectomy and gender differences respectively (Dunn comparisons, $p<0$ 05) Separate groups of animals were tested at each dose point for each group sham-operated males (n = 4–8 rats), castrated males (n = 3–10 rats), sham-operated females (n = 11–22 rats), ovariectomized females (n = 5–11 rats)

to the observations that castration of male rats reduced analgesia to the levels of sham-operated females following systemic morphine analgesia (9) and following both opioid and nonopioid forms of swim analgesia (42) Although ovariectomized rats displayed significantly smaller magnitudes of central morphine analgesia than sham-operated female rats, regression analyses of sham-operated and gonadectomized females failed to reveal significant differences in potency Finally, although estrous phase also induced small, significant, and individual changes in analgesic magnitude following central morphine, the regression analysis again failed to reveal significant differences in potency Female rats in proestrous appeared to display greater magnitudes of peak and total analgesia on the tail-flick and jump tests than the other female groups at the higher (20 and 40 μg) morphine doses A previous report (3) indicated increased sensitivity to systemic morphine analgesia during di-estrous, but comparisons are difficult since their definition of sensitivity was not adequately defined in terms of either magnitude and/or potency The failure to observe consistent estrous and female gonadectomy differences in central morphine analgesia also contrasts with elimination of an opioid form of stress-induced analgesia by ovariectomy and the sensitivity of this form of analgesia to estrous phase and steroid replacement (43) While female rats in di-estrous displayed equivalent magnitudes of both opioid and nonopioid forms of stress-induced

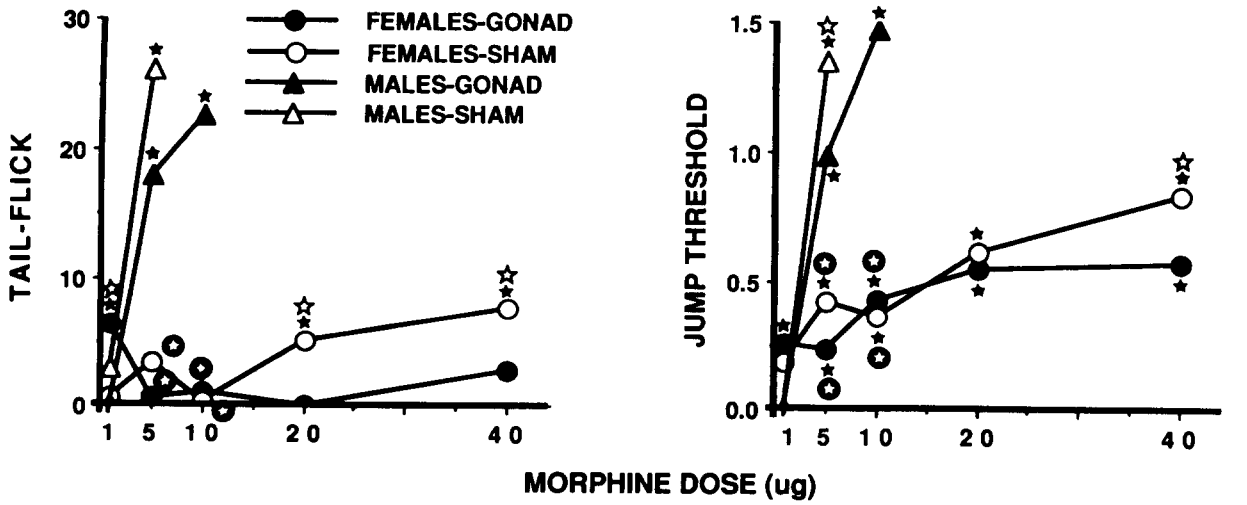

FIG 4 Alterations in total analgesia following intracerebroventricular administration of morphine on the tail-flick and jump tests as functions of gender and gonadectomy The closed stars denote significant differences between the experimental and control values (Dunnett comparisons, $p<0$ 05) The open stars and enclosed stars denote significant gonadectomy and gender differences respectively (Dunn comparisons, $p<0$ 05)

analgesia as males, opioid stress-induced analgesia was reduced during estrous A major difference between this and the present study is a respective reliance upon endogenous (activated by stress) versus exogenous (activated by morphine) mediation of the opioid system

The major gender and smaller gonadectomy and estrous differences in central morphine analgesia could not be attributed to basal shifts in nociceptive reactivity since a) the difference score analyses factored out basal effects, b) basal effects were not observed for vehicle thresholds and c) minimal (less than 1 sec) latency differences were observed in females across the estrous phases. Further, the general lack of gonadectomy differences in central morphine analgesia relative to the observed gonadectomy differences in opioid and nonopioid swim analgesia (42,43) appear to be due to the analgesic procedure employed since the reductions in accessory gonadal tissues across studies (42) were comparable The consistency of the reported effects for central morphine analgesia appeared to vary as a function of the nociceptive measure Although both sham-operated and castrated male rats showed rapidly accelerating dose-response curves for central morphine analgesia on both nociceptive measures, both sham-operated and ovariectomized female rats displayed less variability in the dose-response functions on the jump test than on the tail-flick test Given the intracerebroventricular route of administration, it is possible that these test differences in apparent sensitivity may reflect the level of the neuraxis at which the drug may be acting While the jump test is considered to be a supraspinal reflex (13), the tail-flick test appears to be mediated in part through a spinal component (17,19)

The mechanism mediating gender differences in central morphine analgesia is unknown, but one candidate would be either direct or indirect interactions between central opiate receptors and central gonadal steroid receptors In the original delineation of opiate receptor heterogeneity, the mu receptor subtype was defined in terms of its intrinsic binding characteristics with morphine (29) However, morphine also binds to delta receptor subtypes, although with less affinity than enkephalins (27) Autoradiographic localization of mu and delta receptors reveal an extensive supraspinal (mu > delta) and spinal (delta > mu) distribution (1,28) Central supraspinal morphine analgesia appears to be mediated through supraspinal mu receptors (8, 14, 21) Autoradiographic analysis of gonadal steroid receptors using ^{3}H-estradiol and ^{3}H-testosterone reveal steroid-containing cells in the medial preoptic area, anterior and ventromedial hypothalamic areas, arcuate nucleus, lateral septum, bed nucleus of the stria terminalis, medial and cortical amygdaloid nuclei and the mesencephalic central gray (35,38) In postulating sites of action for direct central effects between opiate and steroid receptors, the three leading candidates would be the mesencephalic central gray, the arcuate nucleus and the amygdala. The mesencephalic central gray is among the most sensitive supraspinal sites to support analgesia [see reviews, (1,51)] The arcuate nucleus contains cells of the medial-basal hypothalamic proopiomelanocortin system which synthesizes beta-endorphin and projects to mesencephalic, metencephalic and myelencephalic loci involved in pain inhibition (1, 28, 50) Combined autoradiography with ^{3}H-estradiol and immunocytochemistry with beta-endorphin reveal cellular co-localization of gonadal target receptors on beta-endorphin-containing cells (22) The amygdala is rich in mu and delta opiate receptors (1,28), and has been shown to support analgesia following microinjection of morphine (39) The mechanisms by which gonadal steroids may act to modulate endogenous opioid pain inhibition are not known, but interactions between gonadal steroids and endogenous opioids have been observed Female rats display lower levels of beta-endorphin (36), dynorphin (34) and Met-enkephalin (20), and these endogenous opioids change across the estrous cycle (20,25) Castration decreases the number of brain opioid receptors (18), pituitary beta-endorphin (48), and pituitary Met-enkephalin (20). Gender differences have also been observed for the distribution and amount of opiate receptor binding (32,33). Although the medial preoptic area of the hypothalamus is not considered a primary site for the mediation of opiate analgesia, its subdivisions like the sexually dimorphic nucleus is larger in males than in females (16) Several neurotransmitters involved in opiate analgesia [see reviews, (30,31)] display gender-specific patterns in the preoptic area Serotonin fibers are most dense in the lateral part of the medial preoptic nucleus which is proportionally larger in females (45) A greater density of tyrosine hydroxylase immunoreactive cells and fibers, but not dopamine beta hydroxylase-immunoreactive cells and fibers, are found in the anteroventral periventricular preoptic nucleus of female rats than male rats, implicating a sexual dimorphism for dopamine (46) Similarly, Met-enkephalin immunoreactivity is also much denser in the anteroventral periventricular nucleus of female rats which is regulated by ovariectomy and neonatal testosterone treatment (49) Simerly and co-workers (44) also found that antisera directed against leucine-enkephalin produced denser immunoreactivity in this nucleus in females than in males, but that antisera directed against peptide E which does not cross-react with dynorphin, produces denser immunoreactivity in males than in females This effect was sensitive to gonadectomy and selective in that antisera directed against either beta-endorphin or dynorphin B displayed gender-insensitive immunoreactivity These data indicate clear gender differences in the organization of opioid peptides in these gonadal steroid-sensitive nuclei Whether this is the site of action for the opioid-sensitive gender differences and/or whether other opioid-containing loci display sexual dimorphism remain to be clarified

In summary, analysis of gender, gonadectomy and estrous effects upon central morphine analgesia revealed a striking gender difference in that females displayed significantly less analgesic magnitude and potency relative to males Although gonadectomy and estrous phase reduced the magnitude of central morphine analgesia at some doses, the potency of morphine analgesia was unchanged by these variables

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