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Gonadal Hormone Modulation of Mu, Kappa, and Delta Opioid Antinociception in Male and Female Rats

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Abstract

Previous studies suggest that sex differences in morphine antinociception in rodents might be attributed to the activational effects of gonadal hormones. The present study determined whether hormonal modulation of opioid antinociception in adult rats extends to opioids other than the prototypic mu agonist morphine. Male and female rats were sham-gonadectomized (sham-GDX) or gonadectomized (GDX) and replaced with no hormone, estradiol (E2, females), progesterone (P4, females), E2+P4 (females), or testosterone (males). Approximately 28 days later, nociception was evaluated on the 50°C hot plate and warm water tail withdrawal tests before and after subcutaneous administration of hydromorphone, buprenorphine, U50,488, or SNC 80. In sham-GDX (gonadally intact) rats, the mu agonists and U50,488 were less effective in females than in males in at least one nociceptive test, and the delta agonist SNC 80 was less effective in males than in females. In males, gonadectomy tended to decrease, and testosterone tended to increase antinociception produced by 3 of the 4 agonists. In females, gonadectomy and hormone treatment had more variable effects, although E2 tended to decrease mu opioid antinociception. The present results suggest that activational effects of gonadal hormones are relatively modest and somewhat inconsistent on antinociception produced by various opioid agonists in the adult rat.

Perspective: This study demonstrates that reproductive hormones such as testosterone in males and estradiol in females do not consistently modulate sensitivity to the analgesic effects of opioids in the adult organism.

Keywords

Testosterone; estradiol; progesterone; pain; analgesia; sex differences

Accumulating evidence suggests that there are sex differences in the acute antinociceptive effects of opioids in rodents, with males generally displaying greater opioid sensitivity than females.¹⁴ Several variables that might influence the observation of sex differences in opioid antinociception include the efficacy/selectivity of the opioid, the type and intensity of the noxious stimulus, and the genotype of the subject.^{5,13,15,29,36,37,49} Sex differences in opioid

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antinociception have been attributed to the differential organizational and/or activational effects of gonadal steroid hormones in males versus females.

Studies that have examined the organizational effects of gonadal hormones report that neonatal gonadal steroid manipulations (castration of males, androgen administration to females) can eliminate sex differences in morphine antinociception in adult rats.^{12,31} Results of studies examining activational effects of gonadal hormones on opioid antinociception are quite variable. In male rats, gonadectomy increased,^{1,10} decreased,^{43,47,48} or failed to change^{9,11,27,28,30} mu opioid antinociception. Similarly, in females, gonadectomy increased,^{1,28,30,47,48} decreased,⁴ or did not change^{11,28,48} opioid antinociception.

The variability across studies that have manipulated gonadal hormones in adult rats might be due to the wide array of methodologic differences across studies. Nearly every study has used different gonadectomy test intervals, hormone replacement regimens, nociceptive testing procedures, and opioid administration procedures. In a previous study from this laboratory, we examined the ability of gonadal steroid hormones to modulate morphine antinociception by using a gonadectomy test interval and hormone replacement regimen that have been used extensively to examine the reproductive system in male and female rats.^{18,20,47,52} This protocol produced well-documented changes in male and female reproductive behavior and physiology and also altered morphine antinociception in male and female rats.⁴⁷ In male rats, gonadectomy decreased morphine's potency, and chronic treatment with testosterone or the combination of its 2 primary metabolites, estradiol (E2) and dihydrotestosterone, restored morphine's potency to that seen in sham-gonadectomized males. In gonadally intact female rats, morphine was more potent during diestrus-1 and proestrus phases relative to the estrus phase. Gonadectomy increased morphine's potency in females, and chronic treatment with E2 or E2 plus progesterone (P4) decreased morphine's potency to that seen in gonadally intact estrus females. With a similar gonadectomy-nociceptive test interval, another laboratory also found that morphine's potency was increased in gonadectomized females relative to sham-gonadectomized females.⁴⁸ In another study, chronic treatment with E2 also decreased morphine's potency relative to gonadectomized females.⁴⁵ These studies suggest that both testosterone in adult male rats and E2 in adult female rats contribute to the sex difference in morphine antinociception.

Previous studies examining gonadal hormone modulation of opioid antinociception have primarily tested only morphine,^{1,4,10,12,27,28,30,31,43,47} with the exception of one study that included several other mu-selective and mixed action opioids.⁴⁸ The latter study examined the effect of gonadectomy on opioid antinociception, but it did not examine the effects of replacement hormones or estrous cycle phase in gonadally intact females, which are crucial for confirming that effects of gonadectomy are due to loss of particular gonadal steroids. The purpose of the present study was to more fully characterize the modulatory role of gonadal steroid hormones on antinociception produced by a variety of opioids. We examined hydromorphone and buprenorphine, 2 opioids that produce antinociception in rodents via mu opioid receptor activation,^{17,26,33} as well as the kappa receptor-selective opioid, U50,488,^{32,40} and the delta receptor-selective opioid, SNC 80.⁸ Buprenorphine was chosen because it is less efficacious than morphine, and previous studies have shown that sex differences tend to be larger with lower efficacy opioids.¹³ Previous studies suggest that gonadal steroids might modulate morphine antinociception by modulating its metabolism.^{3,44,46} Thus, hydromorphone was chosen because it is structurally very similar to morphine and metabolized similarly.² Gonadal hormones were manipulated in male and female rats via gonadectomy and hormone replacement by using dosing regimens that are reproductively relevant.⁴⁷ In addition, gonadally intact female rats were tested during different phases of the estrous cycle, and 2 nociceptive tests were used to determine the generalizability of gonadal hormone modulation of opioid antinociception.

Material and Methods

All procedures were approved by the Washington State University Institutional Animal Care and Use Committee.

Subjects

Male and female Sprague-Dawley rats (bred in-house from Taconic stock, Germantown, NY) were used. Rats were housed in same-sex pairs, according to gonadal steroid status after surgery (eg, an orchidectomized rat with testosterone replacement was housed with another male in the same treatment group). Male and female animals were housed in separate rooms maintained at $21.5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12:12-hour light:dark cycle (lights on at 6:00 AM). Access to food and water was ad libitum except during testing.

General Procedure

Surgery (gonadectomy or sham-gonadectomy) was performed on rats that were approximately 3 months old, as described previously.⁴⁷ In gonadectomized (GDX) rats, basal nociception and opioid antinociception were tested 28 days after surgery, when endogenous hormone levels and associated reproductive behaviors have been shown to be minimal.^{18,20,52} In sham-gonadectomized (sham-GDX) female rats, antinociceptive testing occurred between 21 and 35 days after surgery so that females in diestrus-1 (sham-D), proestrus (sham-P), and estrus (sham-E) could be identified and tested in approximately equal numbers.

Steroid Hormone Replacement

Chronic steroid replacement was achieved via silicone rubber capsules implanted subcutaneously immediately after gonadectomy. A small incision was made between the shoulder blades, capsules were inserted in the subcutaneous space, and the skin was closed with wound clips. Capsules containing testosterone were prepared in 10-mm lengths; one 10-mm testosterone capsule/100 g of body weight was implanted in male animals. Capsules containing E2 were prepared in 1-mm lengths; one 1-mm capsule/rat was implanted in female animals. We have previously shown that these hormone treatments are sufficient to reinstate normal reproductive behavior and physiology in male and female rats, and that they also significantly alter sensitivity to morphine antinociception.⁴⁷ In terms of sexual receptivity and uterine weight, GDX females replaced with E2 as described in this study are similar to intact females in vaginal proestrus to estrus.⁴⁷ To control for possible effects of capsule implantation, sham-GDX and GDX control rats were implanted with empty capsules (0); males received two 10-mm 0 capsules/rat, and females received one 1-mm 0 capsule/rat.

Some female animals also received P4 treatment. Beginning 4 days after surgery, injections of P4 (500 μg /rat in 0.1-mL volume, subcutaneous) were given every 4 days at approximately 10:00 AM to mimic the preovulatory P4 surge observed in normally cycling females.²² All rats (females and males) not receiving P4 treatment received safflower oil vehicle injections (0.1 mL/rat, subcutaneous) on the same schedule, so that all rats were handled/injected the same number of times. Table 1 summarizes the 10 different treatment groups in the study.

Nociceptive Testing

Nociceptive testing began at approximately 2:00 PM to occur within the period of P4's peak reproductive effect, 4 to 6 hours after injection.²¹ Rats were tested on both the hot plate and warm water tail withdrawal assays (both 50°C) consecutively in that order (tests were approximately 10 seconds apart). To obtain non-drug response latencies (to lick a hind paw or jump off the hot plate, or to withdraw the tail from warm water), each rat was tested on each assay 3 times: first at time 0 (baseline 1) and then again 10 to 15 minutes later (baseline 2);

immediately after this second baseline test, saline was administered (1 mL/kg, subcutaneous), and rats were tested a third time on each assay 10 to 15 minutes later (saline test). Immediately after the saline test, increasing doses of one of the opioid agonists in $\frac{1}{2}$ -log (buprenorphine, SNC 80) or $\frac{1}{4}$ -log (hydromorphone, U50,488) unit increments were administered every 10 (SNC 80) or 15 (buprenorphine, hydromorphone, U50,488) minutes, with nociceptive latencies obtained 10 or 15 minutes, respectively, after each injection. A 10-minute rather than 15-minute inter-injection interval was used for SNC 80 because of its relatively short duration of action.⁸ For instance, 0.1 mg/kg hydromorphone was administered, and hot plate and tail withdrawal latencies were obtained 15 minutes later; immediately after testing, an additional 0.22 mg/kg (total 0.32 mg/kg) was administered, and hot plate and tail withdrawal latencies were obtained 15 minutes later. Testing continued with increasing doses of agonist until the rat reached the cutoff latency on both assays. Cutoff latencies of 80 and 25 seconds were used on the hot plate and tail withdrawal assays, respectively. If a rat did not respond by the cutoff latency, the test was terminated, and the cutoff latency was recorded. Once the rat reached cutoff on one assay, subsequent testing was conducted only on the other assay. Each rat was only tested with only one drug; in other words, separate rats were used to examine hormone modulation of antinociception produced by each of the 4 agonists.

Vaginal Cytology

To further characterize gonadal steroid hormone state in female rats, daily vaginal smears were obtained in sham-GDX female rats beginning on day 21 after surgery. Proestrus was identified by the prevalence (approximately 80% or more of epithelial cells in sample) of nucleated epithelial cells; estrus was identified by the prevalence of dense sheets of cornified epithelial cells; diestrus-1 was identified by the presence of leukocytes and scattered nucleated and/or cornified epithelial cells; diestrus-2 was identified by the relative absence of any cells.²⁴ To keep the stages highly distinct, sham-GDX female animals whose vaginal cytology indicated that they were in transition from one estrous stage to another were not tested. To represent a “normal” sample of females, testing in sham-GDX females was not limited to 4-day cyclers. Thus, the designations of diestrus-1 and diestrus-2 are based on vaginal cytology alone and do not necessarily indicate the first and second day after estrus.

Smears also were obtained in sham-GDX female rats immediately after nociceptive testing. Only data from females that remained in the same stage from before to after testing were included in the analyses.

Data Analysis

Latency to respond in seconds was recorded for each hot plate and tail withdrawal test. Unlike the other opioid agonists tested, U50,488 increased the number of rats that jumped off the hot plate rather than licked a hind paw; furthermore, rats that jumped off the hot plate after a low dose of U50,488 were more likely to immediately jump off the plate on subsequent tests. Therefore data from rats that jumped off the plate more than 2 times during testing with U50,488 were removed from further analyses. This resulted in the deletion of data from approximately 2 rats per treatment group tested with U50,488. No data were deleted from any groups tested with the other agonists.

Baseline response latency was calculated for each rat as the mean of the second baseline test and the saline test; latency from the first baseline test was not used because baseline hot plate latency shortens significantly from the first to second test in females.¹⁵ Baseline response latencies were compared between sham-GDX males and females and within the male, sham-GDX, and GDX female groups by using analysis of variance (ANOVA). Because there were significant differences in baseline response latencies among treatment groups, individual response latencies obtained after each dose of drug were converted to % maximum possible

effect (%MPE): $[(\text{Drug latency} - \text{Baseline latency}) / (\text{Cutoff latency} - \text{Baseline latency})] \times 100$. Cutoff latency was adjusted so that the difference between the mean baseline and cutoff value was the same in all hormone treatment groups given the same drug (eg, for rats tested with hydromorphone, the cutoffs were 52 and 17.5 seconds over baseline on the hot plate and tail withdrawal assays, respectively). These cutoff adjustments equalized the %MPE scale among all hormone treatment groups within each drug, so that comparisons of drug potency among hormone treatment groups were based on the same 0% to 100% scale. From these %MPE data, the estimated dose at which antinociception was 50% (ED₅₀) was calculated for each rat by linear interpolation, generally using 1 point above 50% and 1 point below 50% MPE. ED₅₀ values were not calculated when the %MPE value at the highest dose tested was less than 45%.

ANOVA of ED₅₀ values was conducted to test for potency differences in opioid antinociception among the various treatment groups. When ED₅₀ values could not be calculated in more than one third of the rats in a treatment group, treatment group ED₅₀ comparisons were not conducted (Tables 2 and 3). Analyses of %MPE data also were conducted, because these allow for efficacy comparisons among treatment groups based on all data, which is particularly crucial for lower efficacy agonists (wherein ED₅₀ values cannot always be calculated). To determine whether there were sex differences in opioid antinociception, %MPE and ED₅₀ values were compared between the sham-GDX male group and the 3 groups of sham-GDX females combined (data from sham-GDX females in proestrus, estrus, and diestrus were pooled). When significant, differences between particular sham-GDX female groups and the sham-GDX male group are also noted. To determine whether hormone treatments influenced opioid antinociception, %MPE and ED₅₀ values were compared within the male groups, within the sham-GDX female groups, and within the GDX female groups by using ANOVA. Post hoc tests were conducted with Dunnett test, when comparing multiple treatment groups to a control group (eg, comparing GDX+E2, GDX+P4, GDX+E2/P4 groups to the GDX+0 group); with independent samples *t* tests with a Bonferroni correction, to determine at which dose there were group differences in opioid antinociception; or with the Student-Newman-Keuls test, when there were no a priori comparisons (eg, comparing the different sham-GDX female groups). Significance level was set at $P \leq .06$, on the basis of Fisher's zone of indeterminacy.⁵⁰

Drugs

Hydromorphone (Sigma, St Louis, Mo) was dissolved in saline; saline served as the control vehicle for hydromorphone. Buprenorphine (National Institute on Drug Abuse, Rockville, Md) and SNC 80 (synthesized by Drs J. Folk and K. Rice) were dissolved in lactic acid to which distilled water was added; pH was adjusted to approximately 5.5 with 1 N NaOH. A saline solution of pH 5.5 was used as the control vehicle for buprenorphine and SNC 80. U50,488 (National Institute on Drug Abuse) was dissolved in distilled water; distilled water served as the control vehicle for U50,488. Vehicle and opioid injections were subcutaneous (hydromorphone, buprenorphine, U50,488) or intraperitoneal (SNC 80⁸) in a volume of 1.0 mL/kg. P4 (Steraloids, Wilton, NH) was dissolved in safflower oil; safflower oil served as the control vehicle for this steroid. To administer steroid hormones by constant release subcutaneous capsule, silicone rubber tubing (0.062 inches inner diameter/0.125 inches outer diameter) was filled with crystallized hormone or nothing. Capsules were sealed with silicone rubber adhesive and allowed to dry for a minimum of 24 hours. They were then soaked in 70% ethanol for a minimum of 24 hours before use to test the integrity of the seal,¹⁸ and then they were dried before implantation.

Results

Basal Nociception

Baseline response latencies among rats tested with the different opioid agonists showed no significant interactions between agonist and hormonal status within male groups, sham-GDX female groups, or GDX female groups. Therefore, baseline response latency data from rats tested with the 4 different opioids were pooled for subsequent analysis of hormone effects on basal nociception.

Among gonadally intact (sham-GDX) rats, there were sex differences in basal nociception in the hot plate test. Fig 1 shows that sham-GDX females tested in diestrus-1, proestrus, or estrus had significantly longer hot plate latencies than sham-GDX males ($F_{3,141} = 18.06$, $P < .001$). In contrast to the hot plate, there were no significant sex differences in basal nociception in the tail withdrawal test (Fig 1).

Gonadal steroid manipulations in male rats did not significantly affect basal nociception in the hot plate test (Fig 1); however, in the tail withdrawal test, GDX+T males had significantly longer latencies than GDX+0 males. In sham-GDX female rats, there were no significant effects of estrous stage on basal nociception in either the hot plate or tail withdrawal assays (Fig 1). However, all sham-GDX female groups had significantly longer hot plate latencies than gonadectomized females with no hormone replacement (GDX+0 females) ($F_{3,137} = 7.39$, $P < .001$). In the tail withdrawal test, only sham-GDX females in diestrus had significantly longer response latencies than GDX+0 females. Among the GDX females (Fig 1), GDX+E2 and GDX+E2/P4 females had significantly longer response latencies than GDX+0 females in both hot plate ($F_{3,148} = 14.48$, $P < .001$) and tail withdrawal tests ($F_{3,147} = 7.20$, $P < .001$). GDX+P4 females were not significantly different from GDX+0 females on either test.

Hydromorphone Antinociception

Fig 2 shows the hydromorphone dose-effect curves in the hot plate and tail withdrawal tests in all treatment groups; Table 4 shows the corresponding ED₅₀ values. Comparing gonadally intact (sham-GDX) male versus female animals, analysis of %MPE values indicated that hydromorphone produced less hot plate antinociception (sex $\times$ dose: $F_{4,132} = 2.31$, $P = .06$) and less tail withdrawal antinociception in females than in males (sex $\times$ dose: $F_{4,132} = 3.20$, $P = .02$), particularly at the lower doses (0.32, 0.56 mg/kg). ED₅₀ comparisons showed that hydromorphone was significantly more potent in gonadally intact males than in females in the tail withdrawal test ($F_{1,34} = 4.84$, $P = .04$), but not the hot plate test (Table 4). Gonadectomy eliminated the sex difference in hydromorphone antinociception (Table 4, compare GDX+0 males vs females).

Among male treatment groups, there were no significant differences in ED₅₀ values for hydromorphone in either the hot plate or tail withdrawal tests (Table 4), suggesting that hormonal manipulations in males did not significantly affect hydromorphone's antinociceptive potency. However, analysis of the %MPE values revealed a significant interaction between treatment group and dose on the hot plate test ($F_{8,132} = 2.37$, $P = .02$); specifically, 0.56 mg/kg hydromorphone produced greater hot plate antinociception in GDX+T males than in GDX+0 males (Fig 2).

Among sham-GDX female animals tested in diestrus, proestrus, and estrus, hydromorphone produced essentially identical hot plate antinociception; furthermore, these gonadally intact females were not significantly different from gonadectomized females (GDX+0) in their hot plate response to hydromorphone (Fig 2, Table 4). However, in the tail withdrawal test, analysis of ED₅₀ values revealed that hydromorphone was significantly less potent in estrus females than in GDX+0 females (Table 4) ($F_{3,29} = 3.39$, $P = .03$).

Among GDX female animals, hydromorphone was significantly less potent in GDX+E2 females compared to GDX+0 females in the hot plate test (Fig 2, Table 4) ($F_{3,36} = 4.30$, $P = .01$) and in GDX+E2/P4 females compared to GDX+0 females in the tail withdrawal test (Figure 2, Table 4) ($F_{3,36} = 3.45$, $P = .03$). Analysis of %MPE values confirmed these ED₅₀ analyses, showing that hydromorphone produced significantly less antinociception in GDX females treated with E2 in the hot plate test and in GDX females treated with E2+P4 in the tail withdrawal test, compared to GDX females with no hormone replacement.

Buprenorphine Antinociception

Fig 3 shows buprenorphine dose-effect curves in the hot plate and tail withdrawal tests in all treatment groups; Table 5 shows the corresponding ED₅₀ values. Comparing gonadally intact (sham-GDX) males versus females, there were significant sex differences in buprenorphine antinociception in both hot plate and tail withdrawal tests (Fig 3). On the basis of analysis of %MPE values, buprenorphine produced less hot plate antinociception in females than in males (sex $\times$ dose: $F_{4,136} = 2.91$, $P = .02$); however, buprenorphine's potency did not differ significantly on the basis of ED₅₀ values that could be calculated (Table 5). In the tail withdrawal test, buprenorphine produced less antinociception at the higher doses tested (% MPE analysis, sex $\times$ dose: $F_{4,136} = 6.31$, $P < .001$) and was less potent (ED₅₀ analysis, Table 5: $F_{1,31} = 6.92$, $P = .01$) in gonadally intact females than in males. Gonadectomy eliminated the sex difference in buprenorphine antinociception (Table 5, compare GDX+0 males vs females).

Among the male groups, in the hot plate test, buprenorphine was approximately equipotent, but it produced a slightly greater maximal effect in sham-GDX males than in GDX+0 males (Fig 3, Table 5); however, testosterone treatment in GDX males did not significantly increase buprenorphine's maximal effect at the doses tested. In the tail withdrawal test, buprenorphine produced a significantly greater maximal effect at the highest dose tested in sham-GDX relative to GDX+0 males (Fig 3; $F_{2,28} = 3.87$, $P = .03$); buprenorphine's potency was also significantly greater in sham-GDX relative to GDX+0 males (Table 5) ($F_{2,28} = 4.32$, $P = .02$). Testosterone treatment increased buprenorphine's potency slightly but not significantly in the tail withdrawal test.

Among female rats, neither %MPE nor ED₅₀ analyses revealed any significant effects of estrous stage, gonadectomy, or hormone replacement on buprenorphine antinociception in either the hot plate or tail withdrawal tests (Fig 3).

U50,488 Antinociception

Fig 4 shows U50,488 dose-effect curves in the hot plate and tail withdrawal tests in all treatment groups; Table 2 shows the corresponding ED₅₀ values. Comparing gonadally intact (sham-GDX) males versus females, there were no significant sex differences in U50,488 antinociception in either the hot plate or tail withdrawal assays. Analyses of the %MPE values for intact males and individual sham-GDX female groups revealed a significant interaction between treatment group and dose of U50,488 in the tail withdrawal test ($F_{12,116} = 1.90$, $P = .04$); specifically, 18 mg/kg U50,488 produced significantly less effect in sham-estrus females relative to sham-GDX males (Fig 4).

Among the male groups, hormonal manipulations significantly influenced U50,488 antinociception (Fig 4). Analysis of the hot plate %MPE values revealed a significant interaction between treatment group and dose ($F_{8,80} = 2.92$, $P = .007$); specifically, 5.6 and 10 mg/kg U50,488 produced greater antinociception in sham-GDX males than in GDX+0 males. Potency (ED₅₀) comparisons among the 3 male groups yielded nonsignificant differences (Table 2) ($F_{2,20} = 3.02$, $P = .07$). Similar results were obtained in the tail withdrawal test;

although there were no significant differences in ED₅₀ values among the male treatment groups (Table 2), analysis of %MPE values revealed a significant main effect of treatment group ($F_{2,20} = 5.22$, $P = .02$). Specifically, U50,488 produced greater tail withdrawal antinociception in GDX+T males than in GDX+0 males (Fig 4).

Among the sham-GDX females, U50,488 antinociception did not vary significantly across the estrous cycle in the hot plate test (Fig 4, Table 4). In the tail withdrawal test, analysis of %MPE values yielded a nonsignificant group by dose interaction ($F_{8,96} = 1.90$, $P = .07$). Gonadectomy of female rats decreased U50,488 antinociception on the hot plate ($F_{12,124} = 2.01$, $P = .03$); specifically, 5.6 mg/kg U50,488 produced greater hot plate antinociception in sham-GDX females in diestrus than in GDX+0 females (Fig 4). Analysis of ED₅₀ values confirmed that U50,488 tended to be more potent in gonadally intact females than in GDX females without hormone replacement on the hot plate test ($F_{1,33} = 15.97$, $P = .02$). In contrast, gonadectomy of female rats did not significantly alter U50,488 antinociception on the tail withdrawal test.

Among the GDX females, there were no significant effects of hormone manipulation on U50,488 antinociception in the hot plate test (Fig 4). In the tail withdrawal test, however, gonadal hormone manipulations significantly affected U50,488 antinociception (%MPE analysis: group $\times$ dose, $F_{12,136} = 3.77$, $P < .001$). Specifically, U50,488 produced significantly less tail withdrawal antinociception in GDX+P4 females than in GDX+0 females (Fig 4).

SNC 80 Antinociception

Fig 5 shows SNC 80 dose-effect curves in the hot plate and tail withdrawal tests in all treatment groups; Table 3 shows the corresponding ED₅₀ values. Comparing sham-GDX males versus females, analysis of ED₅₀ values showed that SNC 80 was more potent in females than in males in the hot plate ($F_{1,34} = 4.84$, $P = .04$) and tail withdrawal tests ($F_{1,27} = 4.04$, $P = .06$), on the basis of the ED₅₀ values that could be calculated (Table 3). Analysis of %MPE values, which included data from all sham-GDX male and female rats, showed that SNC 80 produced greater hot plate ($F_{1,34} = 6.90$, $P = .01$) but not tail withdrawal antinociception in females than in males.

Among the male groups, gonadal hormone manipulations did not significantly affect SNC 80 antinociception in the hot plate test (Fig 5, Table 3). In the tail withdrawal test, gonadal hormone manipulations in males did not affect SNC 80's potency on the basis of the ED₅₀ values that could be calculated (Table 3). A comparison of %MPE data from all male rats showed, however, that SNC 80's maximal effect was significantly diminished in GDX+T males relative to GDX+0 males (Fig 5; treatment group $\times$ dose: $F_{6,78} = 4.59$, $P < .001$).

Within the sham-GDX female groups, SNC 80 antinociception did not differ significantly among females tested in diestrus, proestrus, and estrus in either nociceptive test (Fig 5, Table 3). However, in the tail withdrawal test, gonadectomy decreased SNC 80's potency in female rats; ED₅₀ values were significantly higher in GDX+0 females than in sham-GDX females (Table 3) ($F_{1,27} = 8.75$, $P = .006$).

Among GDX females tested in the hot plate test (Fig 5), gonadal hormone manipulations modulated SNC 80's potency ($F_{3,29} = 3.10$, $P = .04$) and maximal effects at the doses tested (treatment group $\times$ dose, $F_{9,96} = 2.35$, $P = .02$). However, none of the post hoc tests were significant, so it is difficult to draw conclusions about the specific hormone effects. For example, P4 appeared to increase the potency of SNC 80, but when combined with E2, maximal effect was decreased. In the tail withdrawal test (Fig 5), analysis of %MPE values also showed that there were significant differences in SNC 80 antinociception among GDX female groups ($F_{3,32} = 4.64$, $P < .01$), yet post hoc analyses yielded no differences between specific hormone-treated groups and the GDX+0 group.

Discussion

Hormonal Modulation of Nociception

The present study demonstrates sex differences in latency to respond in the hot plate test but not in the tail withdrawal test. As we reported previously,⁴⁷ female animals had longer hot plate latencies than males, and this sex difference appeared to depend on the presence of E2. Whereas gonadectomy and testosterone replacement in males did not significantly affect hot plate latencies, gonadectomy in females shortened hot plate latency, and E2 replacement, with or without P4, reinstated the longer latencies seen in gonadally intact females. Hormonal modulation of nociception in females was similar in the tail withdrawal test, with gonadectomy shortening and E2 lengthening tail withdrawal latencies. In contrast, in males, testosterone significantly lengthened tail withdrawal latencies despite the fact that gonadectomy had no effect. Previous studies have reported similar gonadectomy and testosterone replacement effects on nociceptive responses in male rats,^{25,25,34,42} and gonadectomy/ovarian hormone replacement effects in female rats,^{23,51} whereas other studies report no apparent hormonal modulation of nociceptive thresholds.^{7,11,30,35,41} It should be noted that we also have observed no hormonal modulation of thermal nociception when using higher intensity nociceptive stimuli than those used in the present study (unpublished data).⁴⁷

Sex Differences in Opioid Antinociception

Table 6 summarizes the present results on sex differences in and hormonal modulation of opioid antinociception and compares these to results we previously obtained by using similar procedures to evaluate hormonal modulation of morphine antinociception in male and female rats.⁴⁷ In the present study, agonists that are known to produce antinociception by acting preferentially at mu opioid receptors (hydromorphone, buprenorphine) were more potent and/or efficacious in gonadally intact males than in females, similar to what we and others have reported previously.^{13,15,17,47,49} The opioid agonist that is known to act preferentially at kappa opioid receptors, U50,488, was also more effective in males than in females in the tail withdrawal but not hot plate test; this result also agrees with previous studies from our laboratory^{15,17} as well as another.⁵ In contrast to the mu and kappa agonists, the delta receptor–preferring agonist SNC 80 produced greater antinociception in females than in males; this sex difference was most reliable on the hot plate, because it occurred when all data were included in the analysis. This result contrasts with our previous studies showing that delta agonists are either equipotent in male and female rats or more effective in males.^{6,17} We can offer no plausible explanation for this discrepancy at present.

Hormonal modulation of antinociception was similar across opioid agonists in some ways but not others. Table 6 shows that in male animals, removal of gonadal steroids via orchidectomy significantly decreased opioid antinociception and/or testosterone replacement significantly increased opioid antinociception for all 3 mu agonists and U50,488 in at least one nociceptive test. For these 4 opioids, the nonsignificant effects of gonadectomy and testosterone replacement showed trends toward the same results. Only SNC 80 antinociception appeared to be modulated differently by testosterone in male rats; in the tail withdrawal test, testosterone decreased rather than increased antinociception produced by SNC 80. Thus, testosterone modulation of opioid antinociception in male rats appears to be similar for mu and kappa opioids, but not for delta opioids.

As described in a recent review article,¹⁶ gonadal hormone modulation of opioid antinociception in adult animals has been examined in approximately 20 previous studies. All but 2 of these studies were conducted in rodents, and nearly all examined only morphine (or stress-induced opioid antinociception). In adult males, gonadal hormones were found to modulate opioid antinociception in 10 of 16 studies; of these 10, gonadal hormones most often

enhanced opioid antinociception.¹⁶ Thus, the present results generally agree with previous studies in that gonadal hormones most often enhanced or did not significantly affect opioid antinociception in male rats. The single exception was for the delta agonist SNC 80; to our knowledge no selective delta agonists have been examined previously in hormone-manipulated males of any species, so it will be important to determine whether antinociception produced by other delta agonists is similarly attenuated.

Table 6 shows that in gonadally intact female animals, antinociception varied significantly across the estrous cycle only in rats treated with morphine; furthermore, the effects of ovariectomy and hormone replacement were quite variable across the 5 opioid agonists examined. Whereas ovariectomy significantly increased antinociception produced by morphine and hydromorphone, it significantly decreased antinociception produced by U50,488 and SNC 80 on at least one nociceptive test and had no significant effect on buprenorphine antinociception. Treatment of ovariectomized females with E2 and/or P4 also produced inconsistent modulation of opioid antinociception, although the effects of morphine and hydromorphone—the most closely related agonists, chemically and pharmacologically—were both decreased by E2 treatment (with or without P4). Examination of the other (nonsignificant) cases showed no consistency in the pattern of hormonal modulation of antinociception in female rats. Although the regimen of E2 replacement in this study was chronic and therefore did not mimic the constantly fluctuating estrogen levels in gonadally intact females, the fact that there were no significant changes in opioid antinociception among sham-GDX females tested in different stages of the estrous cycle further suggests that estrogen might not robustly affect antinociception produced by opioids other than morphine.

A recent review that included hormone modulation of opioid antinociception in females showed that in cycling female rodents, opioid (mostly morphine) antinociception varied significantly across estrous stage in 5 of 7 studies, and comparisons of GDX females to intact or hormone-replaced adult females yielded significant gonadal hormone modulation of opioid (mostly morphine) antinociception in 14 of 19 studies.¹⁶ Although not all hormone effects were in the same direction, 10 of the 14 studies reported that gonadally intact or estradiol-replaced females were less sensitive to opioid antinociception than GDX females.¹⁶ Thus, the present study agrees with previous studies showing that when gonadal hormones do significantly affect mu opioid antinociception in adult female rodents, they are more likely to attenuate rather than enhance it.

There are very few previous studies examining gonadal hormone modulation of antinociception produced by multiple types of opioid agonists. In rats, gonadectomy generally decreased opioid antinociception in males and increased it in females, but this effect depended on opioid efficacy (larger effects for lower efficacy opioids), rat genotype (larger effects in F344 than in Sprague-Dawley strain), and intensity of the nociceptive stimulus.⁴⁸ In fact, the magnitude of the gonadectomy effect clearly paralleled the magnitude of the sex difference in this previous study, suggesting that the small, inconsistent gonadectomy and hormone effects in the present study might simply reflect the relatively small sex differences observed in gonadally intact rats under the testing conditions we used. In the other previous studies conducted in ovariectomized female rhesus monkeys,^{38,39} the effects of gonadal hormones were opposite to those reported in many rodent studies; estradiol and/or progesterone enhanced antinociception produced by some opioid agonists, including butorphanol, nalbuphine, and U50,488, but did not affect antinociception produced by morphine or fentanyl. Enhancement rather than attenuation of opioid antinociception by gonadal hormones is rarely observed in female rodents, suggesting that there might be species differences in gonadal hormone modulation of opioid antinociception.

Taken together, the results of the present and previous studies suggest that testosterone can increase adult male rats' sensitivity to mu and kappa opioid antinociception, but the modulatory effects of E2 and P4 in adult female rats might depend on the specific opioid agonist. The findings further suggest that the existing literature demonstrating gonadal hormone modulation of morphine antinociception in both male and female rodents¹⁶ might not extend to all types of opioid agonists. Of course, examining a greater variety of opioid agonists and nociceptive end points will be necessary to fully test this hypothesis. In addition, factors such as rodent genotype (strain), opioid efficacy, intensity of the noxious stimulus used in the nociceptive test, and dose/duration of hormone treatment have all been identified as important variables in research on sex differences in opioid antinociception,^{14,16} and it will be important to consider these variables when designing future studies on hormonal modulation of nociception. Overall, the results of the present and previous studies suggest that the gonadal steroid hormones testosterone (in males) and estradiol (in females) have relatively modest effects on opioid analgesia in the adult organism, and that sex differences in opioid analgesia might be more readily explained by organizational effects of gonadal steroids.^{12,31}

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Figure 1.

Effects of estrous stage, gonadectomy, and hormone replacement on basal nociception in the 50°C hot plate test (upper panels) and 50°C warm water tail withdrawal test (lower panels). Each bar is the mean $\pm$ 1 standard error of mean (SEM) of 21-28 rats. Treatment group abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus. *Significantly different from GDX + 0 of same sex, $P \leq .05$. #Significantly different from sham males, $P \leq .05$.

Figure 2.

Effects of estrous stage, gonadectomy, and hormone replacement on hydromorphone antinociception in the 50°C hot plate test (upper panels) and 50°C warm water tail withdrawal test (lower panels). Each point is the mean $\pm$ 1 SEM of 7-14 rats. Treatment group abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus. *Significantly different from GDX + 0 of same sex, $P \leq .05$.

Figure 3.

Effects of estrous stage, gonadectomy, and hormone replacement on buprenorphine antinociception in the 50°C hot plate test (upper panels) and 50°C warm water tail withdrawal test (lower panels). Each point is the mean $\pm$ 1 SEM of 8-12 rats. Treatment group abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus. *Significantly different from GDX + 0 of same sex, $P \leq .05$.

Figure 4.

Effects of estrous stage, gonadectomy, and hormone replacement on U50,488 antinociception in the 50°C hot plate test (upper panels) and 50°C warm water tail withdrawal test (lower panels). Each point is the mean $\pm$ 1 SEM of 5-15 rats. Treatment group abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus. *Significantly different from GDX + 0 of same sex, $P \leq .05$.

Figure 5.

Effects of estrous stage, gonadectomy, and hormone replacement on SNC 80 antinociception in the 50°C hot plate test (upper panels) and 50°C warm water tail withdrawal test (lower panels). Each point is the mean $\pm$ 1 SEM of 8-10 rats. Treatment group abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus. *Significantly different from GDX + 0 of same sex, $P \leq .05$. For visual clarity, some data points at 3.2 mg/kg (all at or near zero) are not shown.

Table 1.

Treatment Groups

GROUP	SURGERY	STEROID HORMONE CAPSULES	STEROID HORMONE INJECTION	TREATMENT ABBREVIATION
Males				
1	Sham-orchidectomy	Blank	Vehicle	Sham
2	Orchidectomy	Blank	Vehicle	GDX + 0
3	Orchidectomy	T	Vehicle	GDX + T
Intact females*				
4	Sham-ovariectomy	Blank	Vehicle	Sham-D
5	Sham-ovariectomy	Blank	Vehicle	Sham-P
6	Sham-ovariectomy	Blank	Vehicle	Sham-E
GDX females				
7	Ovariectomy	Blank	Vehicle	GDX + 0
8	Ovariectomy	E2	Vehicle	GDX + E2
9	Ovariectomy	Blank	P4	GDX + P4
10	Ovariectomy	E2	P4	GDX + E2P4

Abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus.

* No sham females were tested during diestrus-2 because, in terms of gonadal steroid levels, reproductive behavior, and reproductive physiology, there are few differences between diestrus-1 and diestrus-2.²⁴

Table 2.

ED₅₀ Values (Mean $\pm$ 1 Standard Error of Mean) for U50,488 Antinociception on the 50°C Hot Plate (HP) and Tail Withdrawal (TW) Assays

Males					Females				
Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*	Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*
Sham	7.30 $\pm$ 2.72	5/5	10.61 $\pm$ 1.55	5/5	Sham-All	6.43 $\pm$ 0.81 [†]	27/27	12.51 $\pm$ 1.51	24/27
					Sham-D	5.27 $\pm$ 1.33	10/10	11.56 $\pm$ 3.06	10/10
					Sham-P	7.28 $\pm$ 1.78	8/8	13.37 $\pm$ 1.33	6/8
					Sham-E	6.96 $\pm$ 1.50	9/9	13.05 $\pm$ 3.00	8/9
GDX + 0	11.53 $\pm$ 0.62	9/9	15.85 $\pm$ 2.91	8/9	GDX + 0	10.45 $\pm$ 1.12	8/8	9.76 $\pm$ 2.68	8/8
GDX + T	8.70 $\pm$ 1.58	9/9	9.88 $\pm$ 1.39	8/9	GDX + E2	7.88 $\pm$ 3.08	7/8	11.66 $\pm$ 2.08	7/8
					GDX + E2/P4	6.40 $\pm$ 1.13	6/7	9.73 $\pm$ 1.93 [‡]	6/7
					GDX + P4	8.31 $\pm$ 1.17	13/15		7/15

Abbreviations: ED₅₀, estimated dose at which nociception was 50%; GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus.

* The number of ED₅₀ values that could be calculated/The total number of rats tested.

[†] Hormone effect: significantly different from GDX+0 rats of the same sex, $P \leq .05$.

[‡] Mean ED₅₀ value not calculated because of insufficient sample.

Table 3. ED₅₀ Values (Mean ± 1 Standard Error of Mean) for SNC 80 Antinociception on the 50°C Hot Plate (HP) and Tail Withdrawal (TW) assays

Males			Females		
Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*	Treatment Group
Sham	42.55 ± 11.75	9/9	73.42 ± 7.00	7/9	Sham-All
					Sham-D
					Sham-P
					Sham-E
GDX + 0	50.47 ± 8.71	10/10	65.86 ± 13.47	10/10	GDX + 0
GDX + T	37.31 ± 9.65	8/10	62.67 ± 17.52	7/10	GDX + E2
					GDX + E2/P4
					GDX + P4

Abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus.

* The number of ED₅₀ values that could be calculated/The total number of rats tested.
† Hormone effect: significantly different from GDX + 0 rats of the same sex, $P \leq .05$.
‡ sex difference: significantly different from sham males, $P \leq .05$.
§ Mean ED₅₀ value not calculated because of insufficient sample.

Table 4.

ED₅₀ Values (Mean $\pm$ 1 Standard Error of Mean) for Hydromorphone Antinociception on the 50°C Hot Plate (HP) and Tail Withdrawal (TW) assays

Males					Females				
Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*	Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*
Sham	0.44 $\pm$ 0.06	12/12	0.50 $\pm$ 0.10	12/12	Sham-All	0.50 $\pm$ 0.04	23/23	0.76 $\pm$ 0.07, ^{†‡}	23/23
					Sham-D	0.53 $\pm$ 0.06	7/7	0.78 $\pm$ 0.11	7/7
					Sham-P	0.47 $\pm$ 0.06	7/7	0.63 $\pm$ 0.06	7/7
					Sham-E	0.51 $\pm$ 0.07	9/9	0.85 $\pm$ 0.15 [‡]	9/9
GDX + 0	0.50 $\pm$ 0.05	10/10	0.37 $\pm$ 0.05	10/10	GDX + 0	0.56 $\pm$ 0.07	10/10	0.46 $\pm$ 0.04	10/10
GDX + T	0.40 $\pm$ 0.03	14/14	0.32 $\pm$ 0.05	14/14	GDX + E2	0.85 $\pm$ 0.07*	10/10	0.52 $\pm$ 0.05	10/10
					GDX + E2/P4	0.61 $\pm$ 0.07	10/10	0.73 $\pm$ 0.11 [‡]	10/10
					GDX + P4	0.55 $\pm$ 0.05	7/7	0.44 $\pm$ 0.04	7/7

Abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus.

* The number of ED₅₀ values that could be calculated/The total number of rats tested.

[†] Hormone effect: significantly different from GDX+0 rats of the same sex, $P \leq .05$.

[‡] Sex difference: significantly different from sham males, $P \leq .05$.

Table 5.

ED₅₀ Values (Mean $\pm$ 1 Standard Error of Mean) for Buprenorphine Antinociception on the 50°C Hot Plate (HP) and Tail Withdrawal (TW) Assays

Males					Females				
Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*	Treatment Group	HP ED ₅₀	N*	TW ED ₅₀	N*
Sham	0.12 $\pm$ 0.03	11/11	0.03 $\pm$ 0.01 [†]	11/11	Sham-All	0.15 $\pm$ 0.02	23/25	0.12 $\pm$ 0.02 [‡]	21/25
					Sham-D	0.12 $\pm$ 0.02	6/8	0.11 $\pm$ 0.04	5/8
					Sham-P	0.14 $\pm$ 0.04	8/8	0.15 $\pm$ 0.05	7/8
					Sham-E	0.17 $\pm$ 0.05	9/9	0.10 $\pm$ 0.04	9/9
GDX + 0	0.19 $\pm$ 0.07	9/11	0.10 $\pm$ 0.02	11/11	GDX + 0	0.14 $\pm$ 0.04	10/10	0.08 $\pm$ 0.02	9/10
GDX + T	0.16 $\pm$ 0.06	9/9	0.07 $\pm$ 0.01	9/9	GDX + E2	0.23 $\pm$ 0.06	9/10	0.11 $\pm$ 0.03	10/10
					GDX + E2/P4	0.12 $\pm$ 0.02	12/12	0.14 $\pm$ 0.05	12/12
					GDX + P4	0.21 $\pm$ 0.05	8/10	0.13 $\pm$ 0.07	8/10

Abbreviations: GDX, gonadectomized; 0, no hormone; T, testosterone; E2, estradiol; P4, progesterone; Sham-D, intact females tested during diestrus-1; Sham-P, intact females tested during proestrus; Sham-E, intact females tested during estrus.

* The number of ED₅₀ values that could be calculated/The total number of rats tested.

[†] Hormone effect: significantly different from GDX + 0 rats of the same sex, $P \leq .05$.

[‡] Sex difference: significantly different from sham males, $P \leq .05$.

Table 6.
Summary of Sex Differences in and Hormonal Modulation of Opioid Antinociception

	Morphine [*]	Hydromorphone	Buprenorphine	U50,488	SNC 80
Sex difference					
HP	Male > female [†]	Male > female [‡]	Male > female [‡]	-	Female > male ^{†,‡,§}
TW		Male > female ^{†,‡}	Male > female ^{†,‡}	Male > female [‡]	Female > male ^{‡,§}
Estrous cycle	Diestrus proestus > estrus [†]	-	-	-	-
Ovariectomy					
HP	↑ Antinociception [†]	-	-	↓ Antinociception [‡]	-
TW		↑ Antinociception ^{†,‡}	-	-	↓ Antinociception ^{†,‡}
+ E2					
HP	↓ Antinociception [†]	↓ Antinociception ^{†,‡}	-	-	-
TW		-	-	-	-
+ P4					
HP	-	-	-	-	-
TW	-	-	-	↓ Antinociception [‡]	-
+ E2/P4					
HP	↓ Antinociception [†]	-	-	-	-
TW	-	↓ Antinociception [†]	-	-	-
Orchidectomy					
HP	↓ Antinociception [†]	-	-	↓ Antinociception [‡]	-
TW	-	-	↓ Antinociception ^{†,‡}	-	-
+ T					
HP	↑ Antinociception [†]	↑ Antinociception [‡]	-	-	-
TW	-	-	-	↑ Antinociception [‡]	↓ Antinociception [‡]

Abbreviations: HP, hot plate; TW, tail withdrawal; -, no significant group difference; E2, estradiol; P4, progesterone; T, testosterone; %MPE; % maximum possible effect; ED₅₀, estimated dose at which nociception was 50%.

^{*} From reference ⁴⁷, rats were tested in HP only, but other methods were identical to those used in the present study.

[†] Based on ED₅₀ analysis.

[‡] Based on %MPE analysis.

[§] ED₅₀ comparison based on subset of data, because ED₅₀ values could not be calculated in all cases.