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Effect of Adrenal and Sex Hormones on Opioid Analgesia and Opioid Receptor Regulation

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CANDIDO, J., K. LUTFY, B. BILLINGS, V. SIERRA, A. DUTTAROY, C. E. INTURRISI AND B. C. YOBURN. *Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation.* PHARMACOL BIOCHEM BEHAV **42**(4) 685-692, 1992.—The role of endocrine factors on opioid analgesia (antinociception) and opioid receptors was studied in male and female Swiss-Webster mice. Morphine was more potent in male than in female mice, although this difference appears to be due to greater availability of morphine to the brain in males. Saturation binding studies indicated that the density and affinity of brain μ- and δ-opioid binding sites were equivalent in males and females. Males and females were implanted SC with naltrexone (NTX) or placebo pellets for 8 days, and then the pellets were removed. This treatment increased the density of μ and δ binding sites in brain and increased the potency of morphine for both sexes, although the increase in antinociceptive effects for males was greater than for females. Adrenalectomy (ADX) in male mice increased the potency of morphine and methadone but did not alter the brain levels of either drug. ADX did not alter brain opioid binding of either μ or δ ligands. When male ADX and control mice were treated with NTX, the potency of morphine and brain opioid binding sites were increased equivalently in both groups. Gonadectomy (GDX) in male mice tended to decrease morphine potency, although this was not found to be a very reliable effect. When male GDX and control mice were implanted with NTX, brain opioid binding was increased similarly in both groups, although morphine potency was increased less in GDX mice. Overall, these studies show that sex differences and hormones of the adrenals and gonads in male mice do not alter brain opioid receptors. Furthermore, these factors do not play a role in NTX-induced opioid receptor upregulation. However, all these factors can modify the potency of opioid agonists.

Sex differences Opioids Opioid receptors Adrenalectomy Gonadectomy Morphine Methadone Naltrexone Receptor upregulation Supersensitivity Analgesia

INTERACTIONS between opioids and the endocrine system have been demonstrated in a number of areas. For example, hypophysectomy and adrenalectomy increase the potency of opioid agonists (2,16,23,24,27,36,37,48). Hormones of the sex organs also appear to play a role in opioid effects since opioid potency varies with sex and can be altered by gonadectomy (28,29,42). The effect of pituitary-adrenal hormones and sex hormones on opioid pharmacodynamics suggests that these endocrine factors may alter opioid receptors. While gonadectomy in male rats has been reported to both increase and not effect whole brain opioid binding (5,6,19,20), there is some evidence that female and male sex steroids can modulate opioid receptor density (21,22,35,49,50).

Opioid receptor upregulation is an important and reliable response of opioid receptors to chronic blockade with an opioid antagonist such as naloxone or naltrexone (NTX) (3,32,46,55-58). The increase in opioid receptor density is accompanied by an increase in opioid agonist potency, commonly referred to as supersensitivity [e.g., (44,55)]. The increase in opioid receptor density typically occurs over an 8-day treatment interval and thus represents a fairly rapid dynamic change. However, the factors that might be important in mediating upregulation by opioid antagonists are not well understood. Given that hormonal variables can alter opioid effects and receptors, it is possible that adrenal-pituitary hormones and sex steroids may modify opioid antagonist-induced upregulation and supersensitivity.

In the present studies, we evaluated the role of sex in morphine pharmacodynamics and opioid receptor upregulation. Furthermore, we examined the effect of adrenalectomy

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(ADX) and gonadectomy (GDX) in male mice on opioid pharmacodynamics and receptor upregulation.

GENERAL METHOD

Subjects

Male and female mice (22-24 g) were obtained from Taconic Farms (Germantown, NY) and housed 5-10/cage with free access to food and water. ADX, GDX, and sham operations were performed by the supplier at least 5 days prior to the start of an experiment. Adrenalectomized and sham ADX mice were maintained on 1% NaCl.

Analgesia Assay

The tail-flick assay was used to determine antinociception (analgesia). Briefly, a beam of light was focused on the dorsal tail surface. Light intensity was set so baseline tail-flick latencies ranged between 2-4 s. Mice were tested at the time of peak effect or, in some studies, periodically following drug injection. Testing was terminated and mice were defined as analgesic if they failed to flick by 10 s. All testing was performed in a blind manner.

Brain Opioid Binding

Binding was determined as previously described (56). Mice were killed and whole brain rapidly removed, weighed, and then homogenized in 20 vol ice-cold 50 mM potassium phosphate buffer (pH 7.2). Homogenates were then centrifuged at 20,000 × *g* for 15 min, the supernatant discarded, and the pellet resuspended in 20 vol buffer and centrifuged again. The pellet was resuspended in 20 vol buffer and incubated for 30 min at 25°C, then centrifuged a third time, and finally resuspended in 20 vol buffer. An aliquot (200 μl) of the final homogenate was assayed in triplicate in tubes containing [^{3}H]DAGO or DPDPE (Amersham Corp., Arlington Heights, IL) alone or in combination with 1,000 nM levorphanol. Homogenates were incubated for 90 min at 25°C. Incubation was terminated by the addition of 5 ml ice-cold 50 mM potassium phosphate buffer and samples were filtered (M24R Cell Harvester, Brandel, Gaithersburg, MD) over GF/B glass fiber filters. Filters were washed twice with 5 ml ice-cold buffer, transferred to scintillation vials, and counted. Specific binding was the difference between the total binding determined in the absence of cold ligand and the binding in the presence of the cold ligand.

Brain Morphine, Methadone, and NTX Levels

The brain was rapidly removed, weighed, and homogenized in 4 vol ice-cold 0.1 N HCl. The homogenate was centrifuged for 15 min at 1500 × *g*. The supernatant was removed and frozen (−20°C) until analysis for morphine, methadone, or NTX by radio immunoassay (RIA) (33,52,53).

Drugs

NTX pellets (nominally 30 mg NTX base) and placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). NTX and placebo pellets were cut into approximately two equal halves. Each half NTX pellet was weighed and the average drug content was determined to be 15.7 mg (± 1.3 SD). Pellets were wrapped in nylon mesh before SC implantation in the nape of the neck while mice were lightly anesthetized with halothane : oxygen (4 : 96). Pellets were removed 8 days following implantation. Morphine sulfate was obtained from Penick Laboratories (Newark, NJ); methadone was obtained from Sigma Chemical Co. (St. Louis, MO). Drugs were dissolved in saline and administered SC. All doses are expressed as the base.

Data Analysis

Quantal dose-response curves were analyzed by the method of Finney (14) using a computerized Probit Analysis program (Bliss 21) that estimates ED_{50} values, relative potencies, and 95% confidence intervals. Saturation binding studies were analyzed using LIGAND for the PC (39).

EXPERIMENT 1: SEX DIFFERENCES IN MORPHINE ANALGESIA AND NTX-INDUCED EFFECTS

Procedure

Male and female mice were injected with morphine (males: 2.5 mg/kg; females 4.0 mg/kg) and tested for analgesia periodically for 2 h (n = 6-7/sex/time point). In a separate study, male and female mice (n = 12/sex) were injected with morphine (4 mg/kg) and the brain removed 45 min later and assayed for morphine by RIA. Male and female mice were also implanted with a 15-mg NTX or placebo pellet for 8 days and brain levels of NTX were determined on the eighth day of implantation (n = 7-10/sex). Other mice (n = 7-10/sex/treatment/time point) were tested for analgesia with the pellet in place following 10 mg/kg morphine 1 or 8 days following implantation. In a separate group of mice, the pellets were removed on the eighth day and 24 h later mice were examined in binding studies or injected with morphine (0.5-7.5 mg/kg, n = 5-17/dose/treatment) and tested for analgesia.

Results

The time course and magnitude of morphine analgesia in male and female mice was not significantly different, $Fs \geq 1.21$, $p > 0.05$, even though in this study the females received a dose that was 60% greater than the males (Fig. 1). For both sexes, analgesia peaked at 30-45 min following morphine and was virtually gone by 2 h. When males and females were in-

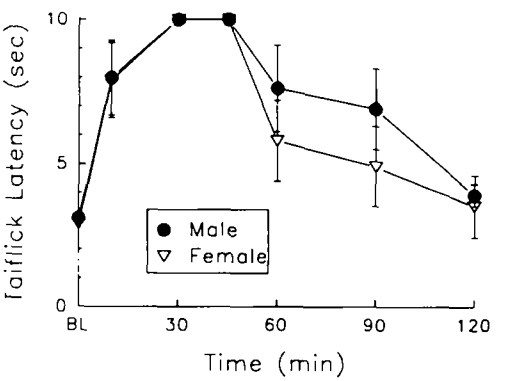

FIG. 1. Effect of sex on the time course of morphine analgesia. Mice (n = 6-7/sex/time point) were injected with morphine (male: 2.5 mg/kg; female: 4.0 mg/kg, SC) and tested for analgesia (tail-flick) periodically for 2 h. Data are the mean tail-flick latency ± SEM.

jected with the same dose of morphine (4 mg/kg), the brain morphine level 45 min later was significantly ($p < 0.05$) greater in males (109.4 ng/g ± 7.1 SEM) than females (80.6 ± 7.5 ng/g).

Male and female mice implanted with placebo and tested for morphine analgesia (10 mg/kg) 1 or 8 days following implantation were all analgesic on both days. Conversely, none of the mice implanted with NTX and tested with the pellet in place were analgesic following morphine on either day. Brain NTX levels 8 days following implantation were not significantly different ($p > 0.05$) for males (62.2 ± 2.9 ng/g) and females (59.9 ± 11.2 ng/g).

In mice that were implanted with pellets for 8 days and then had the pellets removed, followed by analgesia testing 24 h later, male mice were consistently more sensitive to morphine than female mice (Fig. 2, Table 1). Morphine was approximately 45% more potent in male placebo-treated mice compared to female placebo-treated mice. When male mice were treated with NTX, the potency of morphine was increased by 1.85-fold, whereas it was increased significantly ($p < 0.05$) less in female mice (1.39-fold). We observed similar results in three other studies [mean NTX-induced potency shift = 1.98 (±0.17 SEM) males; 1.37(±0.11) females; $p < 0.05$]. In binding studies, NTX increased binding by approximately 50% (DAGO) and 35% (DPDPE). However, there were no differences between males and females treated with placebo or NTX in whole brain [^{3}H]DAGO or [^{3}H]DPDPE binding (Fig. 3).

EXPERIMENT 2: EFFECT OF ADRENALECTOMY IN MALE MICE ON OPIOID ANALGESIA AND NTX-INDUCED EFFECTS

Procedure

ADX and sham male mice were injected with morphine (0.5–5 mg/kg, n = 9–10/dose/treatment) or methadone (0.25–1.0 mg/kg, n = 8–11/dose/treatment) and tested for analgesia 45 (morphine) or 30 min (methadone) later. Separate groups of ADX and sham mice (n = 10/condition) were injected with 4 mg/kg morphine and tested (tail-flick) periodically for 2 h. Morphine (2 mg/kg) and methadone (0.5 mg/kg) levels in brains of ADX and sham mice were determined

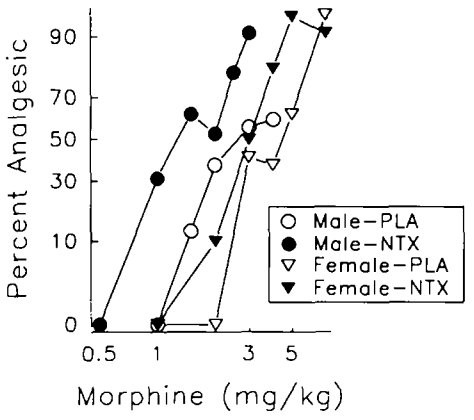

FIG. 2. Effect of sex on morphine analgesia and NTX-induced supersensitivity. Mice were implanted SC with a 15 mg NTX pellet or placebo (PLA) pellet. Eight days later, the pellets were removed and 24 h later mice were injected SC with morphine (0.5–7.5 mg/kg, n = 5–17 dose/treatment) and tested for analgesia (tail-flick) 45 min later (see Table 1).

TABLE 1

EFFECT OF NALTREXONE TREATMENT ON MORPHINE ANALGESIA IN MALE AND FEMALE MICE

Treatment	ED_{50} (mg/kg)	Relative Potency
Male		
Placebo	2.79 (2.33–3.39)	1.00
Naltrexone	1.51 (1.26–1.79)	1.85*
Female		
Placebo	4.06† (3.44–4.79)	1.00
Naltrexone	2.92† (2.42–3.47)	1.39*‡

Mice were treated as described in Fig. 2. ED_{50}s (95% confidence limits) and relative potencies were determined by probit analysis.

*Significant change in potency relative to corresponding placebo group ($p < 0.01$).

†Significantly different ED_{50} compared to corresponding male group ($p < 0.01$).

‡Significantly different from potency change for males ($p < 0.05$).

30 (methadone) or 45 min (morphine) following administration (n = 6–10/treatment). In another study, male ADX and sham mice were implanted with NTX or placebo pellets for 8 days. The pellets were removed and 24 h later mice were tested for morphine analgesia (0.5–4 mg/kg, n = 5–10/dose/treatment) in dose-response studies or the brain was removed and saturation binding studies (DAGO) performed.

Results

ADX increased ($p < 0.05$) the potency of morphine [ED_{50}s (95% limits) = 3.11 mg/kg (2.49–3.87), 1.54 (1.16–1.97), sham and ADX, respectively) (Fig. 4)]. Although morphine was more potent in ADX mice, the time-action profile (not shown) was similar to that of sham-treated mice with peak effect occurring between 30–60 min following injection. ADX also significantly ($p < 0.05$) enhanced the potency of methadone [ED_{50} (95% limits) = 0.79 mg/kg (0.58–1.22), 0.51 (0.35–0.70), sham and ADX, respectively) (not shown)]. However, ADX did not significantly alter the brain levels of morphine (sham = 38.5 ± 7.1 ng/g; ADX = 40.5 ± 2.2) or methadone (sham = 66.6 ± 2.9; ADX = 68.4 ± 5.2).

When sham and ADX mice were treated with NTX, morphine potency was increased by approximately 1.6-fold in both groups (Table 2, Fig. 5). Thus, ADX did not alter the magnitude of NTX-induced supersensitivity. Although ADX mice treated with NTX appeared to be more sensitive to morphine than sham mice treated with NTX, this difference did not reach statistical significance ($0.05 < p < 0.10$). In saturation binding studies with DAGO, NTX increased receptor density by approximately 60%. However, ADX did not alter receptor affinity or density in placebo- or NTX-treated mice. Binding parameters for DAGO were: K_ds = 1.5 (±0.2 SEM), 1.8 (±0.3), 1.6 (±0.3), and 1.7 (±0.1) nM; B_{max}s = 9.4 (±0.7), 15.3* (±0.9), 8.7 (±0.8), and 13.7* (±0.4) for sham-placebo, sham-NTX, ADX-placebo, and ADX-NTX, respectively (*significantly different from sham-placebo, $p < 0.05$).

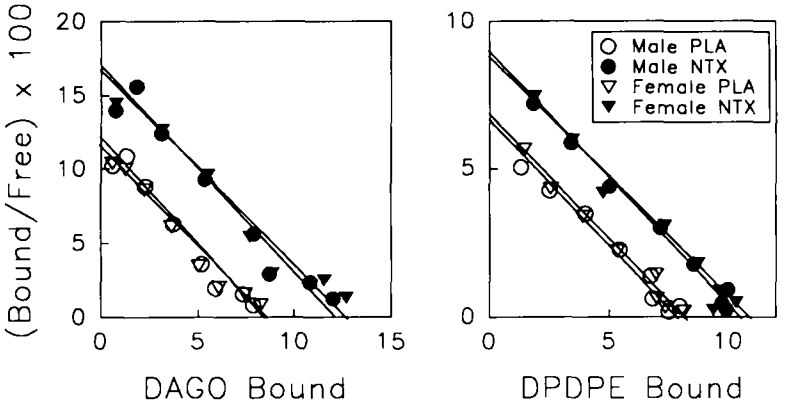

FIG. 3. Effect of sex and NTX treatment on whole brain [^{3}H]DAGO and [^{3}H]DPDPE binding. Mice (n = 3-5/group) were implanted SC with a 15-mg NTX pellet or placebo (PLA) pellet. Eight days later, the pellets were removed and 24 h later mice were killed and saturation binding studies conducted ([^{3}H]DAGO = 0.078-10 nM; [^{3}H]DPDPE = 0.31-40 nM). Binding parameters as determined by LIGAND for DAGO were: K_ds = 0.7 (±0.1 SEM), 0.8 (±0.1), 0.8 (±0.2), and 0.8 (±0.1); B_{max}s = 8.3 (±0.8), 12.7* (±1.3), 8.6 (±1.3), and 13.1* (±1.3),male-placebo, male-NTX, female-placebo, and female-NTX, respectively. Binding parameters for DPDPE were: K_ds = 1.2 (±0.3 SEM), 1.2 (±0.2), 1.2 (±0.1), and 1.1 (±0.2); B_{max}s = 7.8 (±1.1), 10.5* (±1.0), 8.2 (±0.6), and 10.9* (±1.1), respectively; *significantly different from corresponding placebo group ($p < 0.05$).

EXPERIMENT 3: EFFECT OF GDX IN MALE MICE ON MORPHINE ANALGESIA AND NTX-INDUCED EFFECTS

Procedure

GDX (n = 11) and sham (n = 11) mice were injected with morphine (2 mg/kg) and tested over 2 h for morphine analgesia. In a separate study, male GDX and sham mice were implanted with NTX or placebo pellets for 8 days. The pellets were removed and 24 h later mice were tested for morphine analgesia (0.5-4.0 mg/kg, n = 5-19/dose/treatment) in dose-response studies or the brain was removed and binding studies performed.

Results

In pilot studies, GDX tended to decrease the sensitivity to morphine [ED_{50}s (95% limits) = 1.76 mg/kg (1.33-2.19), 2.49 (1.98-3.06) sham and GDX, respectively]. Although morphine was somewhat less potent in gonadectomized mice, the time-action profile following 2 mg/kg was similar to that of sham-treated mice with peak effect occurring 30-60 min following morphine (not shown).

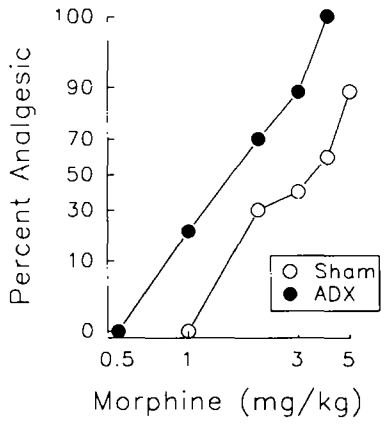

FIG. 4. Effect of adrenalectomy (ADX) on morphine analgesia in male mice. ADX mice and sham-operated controls were injected with morphine (0.5-5.0 mg/kg, SC, n = 9-10/dose/treatment) and tested for analgesia (tail-flick) 45 min later.

TABLE 2

EFFECT OF NALTREXONE TREATMENT ON MORPHINE ANALGESIA IN ADRENALECTOMIZED MICE

Treatment	ED_{50} (mg/kg)	Relative Potency
Sham		
Placebo	2.72 (2.06-3.65)	1.00
Naltrexone	1.65* (1.22-2.11)	1.65†
Adrenalectomy		
Placebo	1.81‡ (1.35-2.33)	1.00
Naltrexone	1.15* (0.85-1.54)	1.57§

Mice were treated as described in Fig. 5. Ed_{50}s (95% confidence limits) and relative potencies were determined by probit analysis.

*Significantly different ED_{50} compared to sham-placebo group ($p < 0.01$).

†Significant change in potency compared to corresponding placebo group ($p < 0.01$).

‡Significantly different ED_{50} compared to sham-placebo group ($p < 0.05$).

§Significant change in potency compared to corresponding placebo group ($p < 0.05$).

Morphine appeared less potent in GDX-placebo-treated mice compared to sham placebo-treated mice, but this did not reach statistical significance (Fig. 6, Table 3). Morphine potency was increased significantly by 2.04-fold in sham NTX

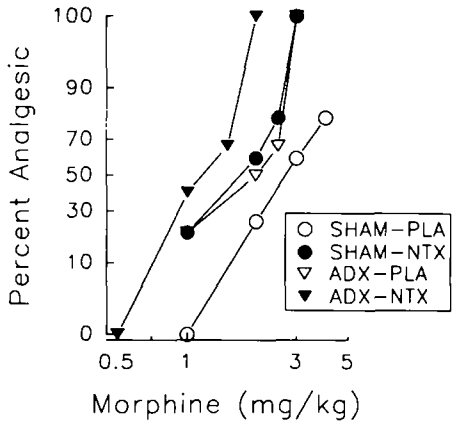

FIG. 5. Effect of adrenalectomy (ADX) on morphine analgesia and NTX-induced supersensitivity in male mice. ADX mice and sham-operated controls were implanted SC with a 15 mg NTX pellet or placebo (PLA) pellet. Eight days later, the pellets were removed and 24 h later mice were injected with SC morphine (0.5–4 mg/kg, n = 5–10/dose/treatment) and tested for analgesia (tail-flick) 45 min later. (see Table 2).

mice (Fig. 6) compared to sham placebo-treated mice. In GDX mice treated with NTX, the potency of morphine increased by 1.46-fold compared to GDX-placebo-treated mice. This increase was significantly ($p < 0.05$) less than that for shams. Finally, NTX similarly (≈60%) increased binding of [^{3}H]DAGO in both GDX and sham mice (Fig. 7). Receptor density was slightly lower, but not statistically different, in GDX mice treated with either placebo or NTX. Similar results on binding were observed when a comparable study was conducted with 2 nM DPDPE (n = 6/group). NTX significantly ($p < 0.05$) increased DPDPE binding by 35 and 37% in the sham and GDX groups, respectively. GDX did not significantly alter DPDPE binding.

DISCUSSION

In the present study, we evaluated the role of sex, ADX, and GDX in male mice on opioid pharmacodynamics and

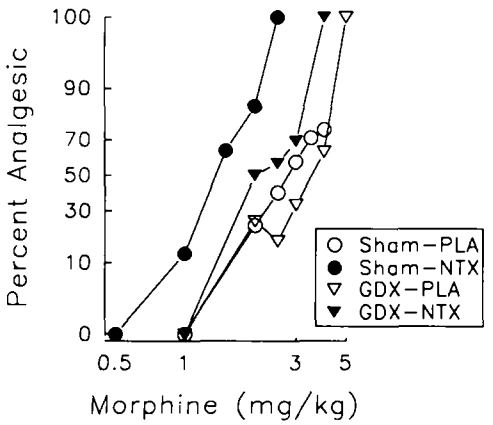

FIG. 6. Effect of gonadectomy (GDX) and NTX treatment on morphine analgesia in male mice. Male GDX and sham mice were implanted SC with a 15 mg NTX pellet or placebo (PLA) pellet. Eight days later, the pellets were removed and 24 h later mice were injected with morphine (0.5–4.0 mg/kg, SC, n = 5–19/dose/treatment) and tested for analgesia (tail-flick) 45 min later. (see Table 3).

TABLE 3

EFFECT OF NALTREXONE TREATMENT ON MORPHINE ANALGESIA IN GONADECTOMIZED MALE MICE

Treatment	ED_{50} (mg/kg)	Relative Potency
Sham		
Placebo	2.81 (2.44–3.23)	1.00
Naltrexone	1.38 (1.17–1.61)	2.04†
Gonadectomy		
Placebo	3.18 (2.73–3.75)	1.00
Naltrexone	2.18 (1.86–2.54)	1.46‡

Mice were treated as described in Fig. 6. Ed_{50}s (95% confidence limits) and relative potencies were determined by probit analysis.

†Significant change in potency compared to corresponding placebo group ($p < 0.01$).

‡Significantly different from potency change for shams ($p < 0.05$).

opioid receptors. Although ADX, GDX, and sex appeared to alter the potency of morphine, and in some cases opioid antagonist-induced supersensitivity, none of these manipulations were found to modify receptor upregulation or receptor affinity. Thus, the functional expression of increases in receptor density may be mediated by endocrine factors, but opioid antagonist-induced receptor upregulation is independent of sex, adrenal, and male gonadal hormones.

As others have shown, we found that ADX enhanced the systemic potency of opioids. We did not find any differences between ADX and controls in the brain concentrations of methadone or morphine. Miyamoto et al. (37) reported that ADX in rats was associated with an increase in CNS morphine concentration following SC administration, although equal brain and spinal cord levels produced greater analgesia in the ADX group. Thus, they concluded that ADX not only increases the access of morphine to the CNS but also increases the sensitivity to morphine. However, when opioids are administered ICV there is no difference in agonist potency between controls and ADX groups (25,37). Taken together, these results suggest that spinal mechanisms may be more important than brain mechanisms in mediating the increase in opioid potency following systemic administration (37).

GDX tended to produce a decrease in morphine potency, although this was not a reliable effect. GDX produced no change in opioid binding in brain either in mice treated with placebo or NTX, a finding consistent with that of Cicero et al. (5,6). Therefore, neither basal opioid receptor density or affinity nor receptor upregulation were affected by depletion of male gonadal hormones. Surprisingly, GDX limited the development of supersensitivity to morphine following chronic NTX treatment. Thus, while receptor density was increased similarly in controls and GDX mice the functional expression of upregulation was modified by GDX. Since we did not measure spinal opioid receptors, it is possible that GDX may have altered upregulation and supersensitivity of spinal opioid mechanisms. Alternatively, the lack of male gonadal hormones may have altered intracellular processes that

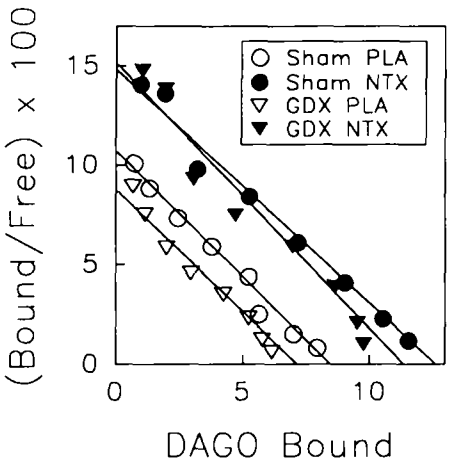

FIG. 7. Effect of gonadectomy (GDX) and NTX treatment in male mice sex on whole-brain [^{3}H]DAGO binding. Male GDX and sham mice (n = 4/group) were implanted SC with a 15-mg NTX pellet or placebo (PLA) pellet. Eight days later, the pellets were removed and 24 h later mice were killed and saturation binding studies conducted [^{3}H]DAGO = 0.078–10 nM). Binding parameters as determined by LIGAND were: K_ds = 0.9 (±0.2 SEM), 0.9 (±0.1), 0.9 (±0.1), and 0.7 (±0.1); B_{max}s = 7.4 (±1.3), 12.2* (±1.1), 6.7 (±0.5), and 10.7* (±1.0), sham-placebo, sham-NTX, GDX-placebo, and GDX-NTX, respectively; *significantly different from corresponding placebo group ($p < 0.05$).

are coupled to some of the new binding sites in NTX-treated mice. This suggestion is supported by our previous studies in which dissociation of upregulation and supersensitivity have been reported (7,13).

Male Swiss-Webster mice were more sensitive to morphine than females. The potency of morphine was approximately 45% greater in males relative to females. This difference did not appear to be due to changes in opioid receptor density or affinity in brain. Similarly, sex differences in opioid potency without differences in binding have also been reported in the rat (29). However, the potency difference in our study may be related to pharmacokinetic considerations. We found that morphine levels in brain were approximately 35% higher in male mice compared to females. When mice were chronically treated with NTX, the density of opioid binding sites in brain increased similarly in males and females. However, while both NTX-treated males and females were supersensitive to morphine the magnitude of supersensitivity was less in females. This may represent a potentiating effect of male gonadal hormones on supersensitivity since GDX males also displayed significantly less NTX-induced supersensitivity than sham-operated controls. It should be noted that NTX levels in brain determined on the eighth day of treatment were equivalent in males and females. However, this does not preclude the possibility that NTX levels earlier in treatment were different in males and females, as was observed for acute morphine treatment. Therefore, female mice may have received a reduced cumulative dose of NTX and this may account for the reduction in supersensitivity. On the other hand, the equal receptor upregulation in males and females suggests that sufficient NTX was received by both sexes.

Our intention in these studies was to evaluate if the hormones of the adrenal and gonads play a role in determining opioid receptor density and upregulation. If receptor upregulation involves synthesis of new receptors, it might be expected that gonadal and adrenal steroid manipulations would alter receptor density. We anticipated this result because glucocorticoids and sex steroids can alter gene expression in a variety of systems. For example, glucocorticoids modulate the levels of preproenkephalin mRNA and enkephalin peptides (4,26,31, 40,54), as well as regulate the density of β-adrenoreceptors and its mRNA (11,12,18). Sex steroids also regulate the levels of endogenous opioid peptides and their mRNAs (1,8,9,30, 38). In addition, sex steroids may regulate opioid receptors (21,22,51) and β-adrenoreceptor density and its mRNA (10,43). Unfortunately, for the opioid receptor we do not have any direct way to measure the effects of these steroids on mRNA until appropriate clones are available.

Adrenal and sex steroids were without effect on opioid antagonist-induced receptor upregulation. Since steroids can modulate gene expression, this finding raises the possibility that opioid antagonist-induced upregulation may not involve synthesis of new receptors. It has been observed that naloxone-induced upregulation in vitro is not blocked by the protein synthesis inhibitor cyclohexamide (45). These investigators suggested that antagonist-induced upregulation may be due to changes in receptor degradation or activation or unmasking of "silent" receptors (45,58). Recently, it has been reported (17,47) that chronic treatment with dopamine antagonists increases dopamine D_2 receptor density but does not alter receptor mRNA levels. This finding agrees with suggestions that antagonist-induced dopamine receptor upregulation may depend upon a change in receptor degradation rate (41). On the other hand, 6-hydroxydopamine lesions of the substantia nigra (receptor denervation) increase dopamine D_2 receptor density and its mRNA (34). These findings are consistent with suggestions that the mechanism of antagonist-induced upregulation is independent of gene expression (17,47,58). Perhaps, opioid receptor upregulation that does not require antagonist treatment (15) may involve gene expression and therefore may be sensitive to sex and adrenal hormones.

In summary, sex and adrenal factors can exert effects on opioid potency. These effects may depend upon changes in sensitivity of opioid systems and to some degree on alteration in pharmacokinetics. Conversely, these endocrine variables do not alter opioid receptors or receptor upregulation.

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