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Sex Differences in Discriminative Stimulus and Diuretic Effects of the κ Opioid Agonist U69,593 in the Rat

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CRAFT, R. M., P. KRUZICH, J. BOYER, J. HARDING AND J. HANESWORTH. *Sex differences in discriminative stimulus and diuretic effects of the κ opioid agonist U69,593 in the rat*. PHARMACOL BIOCHEM BEHAV **61**(4) 395–403, 1998.—Female and male rats were trained to discriminate the κ opioid agonist (5α,7α,8β)-(−)-*N*-methyl-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide (U69,593, 0.13 mg/kg SC) from vehicle using a FR-10 schedule of food reinforcement. Female rats took significantly longer than males to acquire the discrimination (66.9 vs. 44.1 sessions, respectively), and the ED_{50} for U69,593 discrimination was significantly higher in females than in males (0.074 vs. 0.025 mg/kg). The time course of U69,593 discrimination also differed between the sexes: peak and offset occurred earlier in females than in males. The ED_{50} for bremazocine substitution was significantly higher in females than in males (0.0039 vs. 0.0006 mg/kg), whereas ethylketazocine substituted for U69,593 in all males and five of seven females, with no sex difference in substitution ED_{50}. Morphine and BW373U86 did not substitute for U69,593 in a majority of rats of either sex. U69,593 also produced significantly less urine output/dose in females compared to males (e.g., 5.92 vs. 14.83 ml urine/kg body weight after 1.0 mg/kg U69,593), but was equipotent between the sexes in producing hot-plate antinociception. There was no sex difference in response rate-decreasing effect of any opioid agonist tested, and no sex difference in brain/blood ratio of [^{3}H]U69,593 measured in a separate group of rats, suggesting that sex differences observed in some effects of U69,593 probably are not due to sex differences in U69,593 pharmacokinetics. When retested at the end of the study, U69,593 and bremazocine were no longer differentially potent as discriminative stimuli in females and males, suggesting that factors that change over time (e.g., additional training, age, hormonal status) may contribute to initial sex differences in discriminability of U69,593. © 1998 Elsevier Science Inc.

discrimination Opioids U69,593 Sex differences Diuresis

THERE is a growing number of reports on sex differences in the acute behavioral effects of drugs. For example, psychostimulants such as cocaine and amphetamine produce greater increases at a given dose in spontaneous locomotion (15,48), stereotyped behavior (3), and rotational behavior (4) in female rats than in males; moreover, sensitization to some of these effects is greater in females than in males (5,48). Additionally, several investigators have reported sex differences in analgesic effects of opioids (1,7,18,22,23). In contrast, there have been few studies of sex differences in discriminative effects of drugs, which, unlike acute locomotor or analgesic effects, involve learned responses. Perkins and colleagues showed that there were no sex differences in a nicotine discrimination in humans, although women and men may have used different stimuli to discriminate nicotine from placebo (34). Similarly, there were no sex differences in a *d*-amphetamine discrimination in humans (6), and there were few sex differences in a cocaine discrimination in rats (10). Thus, based on the limited published data to date, there are no substantial sex differences in discriminability of psychostimulants. In contrast, we recently reported sex differences in discrimination of the μ opioid agonist morphine in rats, with females acquiring the discrimination in fewer sessions than males did, and morphine being more potent in females than in

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males in producing its discriminative effects (9,11). Although the sex differences in morphine discrimination were primarily differences in potency rather than in magnitude of effect, these results were somewhat surprising given the widely reported result that morphine is a more potent analgesic in males than in females (1,7,21,22); the discrepancy between analgesic and discriminative effects underscores the potentially important distinction between acute (unlearned) and learned effects of drugs.

The purpose of the present study was to determine whether sex differences in discriminative stimulus effects are common to the opioid class of drugs by examining discrimination of a selective κ opioid agonist, U69,593 (24), in female vs. male rats. Currently, there is no clear consensus regarding sex differences in acute effects of κ opioid agonists. For example, at the doses tested, pentazocine and butorphanol produced greater maximal analgesia in women than in men (17,18), and bremazocine produced greater maximal antinociception in female than in male rats on the tail-withdrawal assay (2). In contrast, U50,488 produced greater maximal hot-plate antinociception in male than in female mice (21), and U69,593 produced hot-plate antinociception of approximately equal magnitude in female and male rats (2). There are few studies examining potential sex differences in any other effects of κ agonists. Given the paucity and inconsistency of previous data, and the fact that sex differences in acute drug effects may not predict sex differences in discriminative effects (11), no prediction was made regarding sex differences in discriminative stimulus effects of U69,593.

A number of parameters are used to describe a drug discrimination, including speed of acquisition, dose required to establish a stable, accurate discrimination, duration of the discriminative stimulus effect, and generalization of the discriminative stimulus to other drug stimuli. Thus, to determine whether the U69,593 discriminative stimulus differed between the sexes, gonadally intact female and male rats were compared on rate of acquisition and time course of a U69,593 discrimination, and on patterns of substitution with several opioid agonists. Additionally, drug discrimination rats were evaluated on hot-plate and diuresis tests so that sex differences in physiological vs. discriminative stimulus effects of U69,593 could be compared. Consistent sex differences in all of U69,593's effects would support a hypothesis of sex differences in pharmacokinetics of U69,593, whereas inconsistent sex differences across end points would indicate that some pharmacodynamic factor also differs between the sexes. Brain and blood levels of [^{3}H]U69,593 also were measured in a separate group of naive rats, to more directly examine potential sex differences in pharmacokinetics of U69,593.

METHOD

Subjects

Subjects were male and female Sprague–Dawley rats (Taconic Farms-derived), approximately 4 months old at the beginning of the experiment. Nine rats of each sex were used in the discrimination experiment, and 12 rats of each sex were used in the pharmacokinetics experiment. Rats were singly housed; males and females were housed in separate, adjacent rooms. Rats used in the discrimination experiment were allowed to gain weight gradually during the training phase of the study, so that they would be approximately 85% of age-appropriate free-fed weight by the testing phase of the study (when body weights were held constant at approximately 300 g for females and 500 g for males). To maintain rats at the appropriate body weights, they were fed approximately 2 h post-session Monday through Friday, with three times the daily amount of chow on Friday (for the weekend); water was available ad lib. Vivarium conditions were 72 ± 1°F average temperature and a 12 L:12 D cycle, lights on at 0700 h.

Apparatus

Drug discrimination training and testing were conducted in standard, sound-attenuated, two-lever operant chambers (Med Associates, St. Albans, VT). Half of the chambers were designated for males only and half were designated for females only. Levers were 7 cm above the chamber floor, equidistant from the central food hopper, and required approximately 30 g of force to register a response. There was a stimulus light above each lever, and houselight on the wall opposite the levers. All sessions were controlled and data collected by microcomputer.

Diuretic effects of U69,593 were assessed by placing rats in separate operant chambers that each had a houselight, a stimulus light, and a water bottle but no levers; a wheel manipulandum projected into the chamber and turns of the wheel had no programmed consequences. There were separate chambers for female and male rats. Urine was collected in a stainless steel pan under the 1/4-in. wire mesh chamber floor. Antinociceptive testing was conducted using a hot-plate analgesia meter (Columbus Instruments, Columbus, OH) set at 52 ± 0.1°C. Both female and male rats were tested in the same hot-plate apparatus, which was wiped clean of urine/feces in between tests.

Drug Discrimination Procedure

Rats were initially trained to press levers for 45-mg food pellets (P. J. Noyes, Lancaster, NH) on a continuous reinforcement schedule; the reinforcement schedule was then gradually increased to a fixed ratio (FR)-10 schedule over several weeks. During this training period, only the houselight and the stimulus light above the correct lever were illuminated during the session. Once responding was stable on the FR-10 schedule on both levers (minimum 200 total responses/session for a week), 0.1 mg/kg U69,593 or vehicle was administered SC to six male and six female rats, 15 min presession on a pseudorandom, 5-day/week schedule, such that U69,593 and vehicle were each administered at least twice a week and not more than twice in a row. Rats were placed in the chambers immediately before the session started. Responding on the right lever after U69,593 administration was reinforced in half of the rats of each sex, and responding on the left lever after U69,593 administration was reinforced in the other half of rats of each sex. Beginning 2–3 weeks after initiation of U69,593/vehicle injections, the houselight and both stimulus lights were illuminated during the session, so that the stimulus lights no longer served as a discriminative cue. Responding on the incorrect lever resulted in no reinforcement, and resetting of the FR-10 schedule on the correct lever. Training criteria were 9 of 10 consecutive sessions in which ⩾90% of total responses were made on the correct lever and ⩽5 responses before completion of the first FR-10 were made on the incorrect lever; furthermore, the 1 incorrect session out of 10 could not be the most recent drug or vehicle session. After 6 weeks of 5-day/week training sessions, only 2 of 12 rats (both males) had acquired the discrimination; at that point the training dose was increased to 0.13 mg/kg and the pretreatment time was lengthened to 30 min [the pretreatment time used in previous κ opioid discriminations in rats: e.g., (32,35,41)]. Rats

that had acquired the 0.1 mg/kg U69,593 vs. vehicle discrimination were retrained at the higher training dose until they again met training criteria (at least 10 sessions), before they were tested. Three additional females and three males began training after the new training conditions were imposed; these rats were only trained with 0.13 mg/kg U69,593 using a 30-min pretreatment.

When rats met training criteria, substitution tests were conducted every Tuesday and Friday as long as the most recent U69,593 and vehicle training sessions were correct (≥90% of total responses on the correct lever). During test sessions, responding on each lever was reinforced under a FR-10 schedule (responses on each lever cumulated separately). Training sessions were continued on Mondays, Wednesdays, and Thursdays. Both training and test sessions were 20 min long.

After obtaining the U69,593 discrimination curve, substitution curves were obtained in the following order: bremazocine, morphine, BW373U86, U69,593 time course, ethylketazocine. Within each drug, no rat was tested with the same order of doses. To expedite testing, seven rats/sex were tested with the opioids bremazocine and ethylketazocine, and on the U69,593 time course, and six rats/sex were tested with morphine and BW373U86. To generate the time course of U69,593 discrimination, 0.13 mg/kg U69,593 was administered, and 1, 5, 15, 30, 60, or 120 min later rats were tested for 5 min only (separate test day for each time point). In the same drug-discrimination rats, after these initial substitution tests were completed, antinociception and diuresis tests were conducted (generally on Tuesdays or Fridays, instead of the drug-discrimination session); discrimination training sessions were continued on Mondays, Wednesdays, and Fridays. The U69,593 and bremazocine discrimination curves then were redetermined at the end of the study in most rats to determine consistency of potency differences between female and male rats.

Antinociception Procedure

Thirty minutes after SC injection, rats were placed on a 52°C hot plate. Latency to lick a hindpaw was recorded in seconds, and the rat was immediately removed from the hot plate. If the rat did not make the licking response within 45 s, it was removed from the hot plate and assigned a maximal score of 45 s. Each rat was tested twice with vehicle, 0.1, 0.3, 0.56, and 1.0 mg/kg U69,593 (different order of dosing for each rat), and the mean of the two scores was calculated for each rat at each dose. Antinociception was tested no more than once/week.

Diuresis Procedure

Immediately after SC injection, rats were placed in a dark operant chamber. Urine output was measured in milliliters at 2 h postinjection. Each rat was tested once with saline, vehicle, 0.1, 0.3, and 1.0 mg/kg U69,593 (different order of dosing for each rat).

Pharmacokinetics

Rats were anesthetized with sodium pentobarbital (50 mg/kg IP), and then injected with 8.3 μCi of [^{3}H]U69,593 and 8.3 μCi of [^{14}C]inulin (New England Nuclear, Boston, MA) in physiologic saline (0.5 ml each, SC). Thirty, 60, or 90 min later, rats were decapitated, 0.5 ml of trunk blood was collected, and brains were removed and weighed. Blood and whole brain samples were solubilized in NCS tissue solubilizer (Amersham, Arlington Heights, IL) per manufacturer's instructions for approximately 20 h. After complete solubilization, 100 μl of each sample was counted for radioactivity (Hewlett Packard 1600 TR scintillation counter).

Drugs

U69,593 [National Institute on Drug Abuse (NIDA), Rockville, MD] was dissolved in EtOH to which distilled water was added for a final EtOH concentration of 10% (1 mg/ml stock solution); for lower doses used in the discrimination task, dilutions were made from this stock solution. Thus, the training dose of U69,593 was in a 1.3% EtOH solution, which was also used as the discrimination vehicle. For the diuresis and antinociception tests, all doses of U69,593 were in 10% EtOH, and the vehicle was 10% EtOH. Morphine sulfate (Mallinckrodt, St. Louis, MO) and BW373U86 (gift of Burroughs Wellcome Co., Research Triangle Park, NC) were dissolved in 0.9% physiological saline. Bremazocine (NIDA) and ethylketazocine (Sterling-Winthrop Research Institute, Rensselaer, NY) were dissolved in 85% lactic acid, to which distilled water was added (pH adjusted to 5.5 with 1 N NaOH). All injections were given in volumes of 1.0 ml/kg. U69,593, bremazocine, ethylketazocine, and morphine were administered SC (30, 30, 15, and 20 min presession, respectively, in the discrimination task), whereas BW373U86 was administered IP, 15 min presession. Drug pretreatment times were chosen based on previous drug discrimination studies (11,35).

Statistical Analyses

Percent drug-lever responding (total, and before completion of the first FR) and response rate were recorded daily. Number of sessions to acquire the discrimination was the number of training sessions conducted (without stimulus light cues) that it took for a rat to meet training criteria (see above), at the initial dose of U69,593 (0.1 mg/kg) plus at the subsequent dose of U69,593 (0.13 mg/kg) for rats that were trained on both doses, and just at the higher dose for rats that were trained only at that dose. One male (one of three trained only at the higher dose) died for unknown reasons within the first 2 weeks of training, so no data could be collected from that rat. In addition, data from one female (one of the three trained only at the higher dose) that did not meet training criteria within 105 sessions are not included in any analyses. Percent error during training was the number of sessions in which a rat failed to make ≥90% of total responses on the appropriate lever (with ≤5 responses on the incorrect lever before completion of the first FR) divided by the total number of sessions trained in that condition (until overall training criteria were met); percent error was calculated separately for vehicle and U69,593 conditions. For substitution tests, some doses (generally in the middle of the dose–effect curve) were tested more than once in an individual subject; in these cases, the mean of all tests for a given subject was used in analyses. For each sex, ED_{50} values, 95% confidence limits (C.L.) and slope of the dose–effect curve were calculated by log-linear regression analysis, using data in the linear portion of the curves of all rats of that sex (47). For substitution tests, mean "% responses on the U69,593 lever" included data only from rats that completed at least one FR (obtained at least one reinforcer); however, mean response rate included data from all rats tested. "Complete substitution" of a drug (or U69,593, at doses or pretreatment times other than the training condition)

for the training condition was defined as ⩾80% of total responses made on the U69,593-associated lever.

On the diuresis test, to adjust for individual differences in body weight, urine output (ml) was divided by body weight (kg). In the pharmacokinetics experiment, a brain/blood ratio of [^{3}H]U69,593 was calculated by dividing cpm/g of brain tissue by cpm/ml of blood for each rat; no adjustments were made for blood within brain tissue because [^{14}C]inulin counts in brain were extremely low relative to those in blood (⩽0.5%).

Student's *t*-test, or nonoverlapping confidence limits (for sex differences in one dependent variable) or two-way, repeated measures ANOVA (sex × dose, or sex × time) were used to determine whether sex differences in drug effects were statistically significant. When significant interactions were obtained, post hoc tests were used to determine at which doses or time points the sex difference occurred. Significance level was $p \leqslant 0.05$.

RESULTS

Acquisition of U69,593 Discriminative Stimulus

Only two rats, both males, acquired the 0.1 mg/kg U69,593 vs. vehicle (15-min pretreatment time) discrimination within the first 6 weeks of injections. One male died from unknown causes during the second week of training. When the training dose was increased to 0.13 mg/kg (and the pretreatment time was lengthened to 30 min), all rats acquired the discrimination within 105 sessions except for one female. A comparison of the total number of sessions required to meet training criteria showed that the remaining eight females took significantly longer than the remaining eight males to acquire the discrimination: females took 66.9 ± 27.1 sessions, whereas males took 44.1 ± 17.9 sessions, ($t(14) = 2.19$, $p = 0.05$). For the subset of rats trained only at the higher dose, training took an average of 45 sessions in the two males and 54 sessions in the two females (the third female failed to meet training criteria within 105 sessions). During acquisition, both sexes made slightly more errors in vehicle sessions than in U69,593 sessions: of the rats that met training criteria, females erred in 44 ± 7% of vehicle sessions vs. 39 ± 6% of U69,593 sessions, and males erred in 38 ± 5% of vehicle sessions vs. 26 ± 7% of U69,593 sessions [sex: $F(1, 14) = 7.18$, $p = 0.02$; lever: $F(1, 14) = 1.20$, NS). However, there was no significant sex difference in rate of reinforcement on U69,593 vs. vehicle levers.

Discriminative Stimulus Effects of U69,593

Figure 1 (top panel) shows that the mean U69,593 discrimination curve for female rats was approximately 1/4 log unit to the right of the mean curve for male rats. The ED_{50} value for U69,593 discrimination was significantly higher in female rats compared to males: 0.074 (C.L. 0.057, 0.096) vs. 0.025 (C.L. 0.016, 0.038) mg/kg, respectively. Additionally, potency of U69,593 as a discriminative stimulus was positively correlated with sessions to acquire the discrimination in individual rats (Pearson $r = 0.62$, $p = 0.01$); that is, rats that took more sessions to acquire the discrimination (mostly females) tended to have higher ED_{50} values. The slopes of the dose–effect curves did not differ significantly between males and females. Response rates after saline administration and U69,593 administration also were very similar in females and males; U69,593 produced dose-dependent decreases in response rate in both sexes (Fig. 1, bottom panel). All females except one showed evidence of estrous cycling—at least 1 day of proestrus or estrus in a week of sampling—at this point in the study, as evidenced by vaginal cytology (16).

Figure 2 shows that there was a significant sex difference in the time course of U69,593 discrimination, with onset, peak, and offset of effect occurring somewhat earlier in females than in males [sex × time: $F(6, 72) = 2.77$, $p = 0.02$]. Complete substitution (⩾80% U69,593-lever responding) occurred only at 15 min postinjection in females but at 30 and 60 min postinjection in males; offset of U69,593's effect (⩽20% drug-lever responding) occurred by 90 min postinjection in females but by 120 min postinjection in males. There were no sex differences in response rate over the time course, and no significant changes in response rates over time.

Figure 3 shows substitution patterns for four additional opioid agonists in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. The vehicle used for brema-

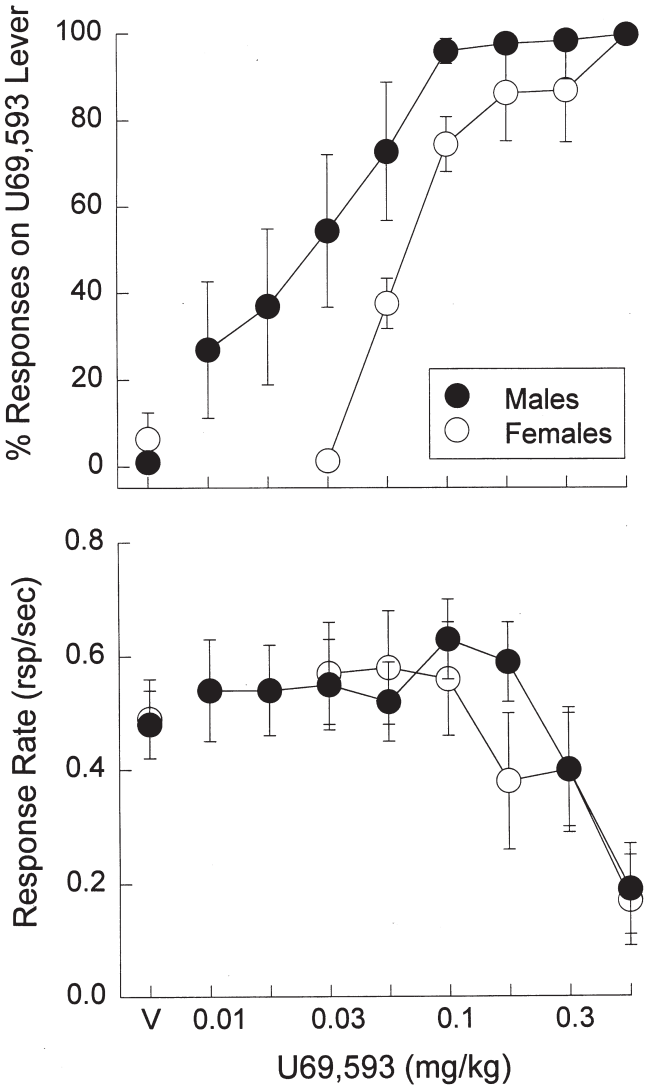

FIG. 1. Discriminative stimulus (top panel) and response rate-altering (bottom panel) effects of U69,593 at the beginning of the study, in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. Injections were given 30 min before the session. Each point is the mean ± 1 SEM of eight females or eight males.

zocine and ethylketazocine (lactic acid/water) did not produce substantial responding on the U69,593-appropriate lever in rats of either sex (upper left panel). The κ agonist bremazocine (left panels) completely substituted for U69,593 in all but one rat, but the ED_{50} for bremazocine substitution was significantly higher in females than in males: 0.0039 (C.L. 0.0029, 0.0051) vs. 0.0006 (C.L. 0.0002, 0.0015) mg/kg, respectively. Bremazocine did not substitute for U69,593 in one male, up to a dose that eliminated responding (0.01 mg/kg, a dose 1/4–1/2 log unit lower than the doses that eliminated responding in all other rats). In addition to the sex difference in potency of bremazocine to substitute for U69,593, the slopes of the bremazocine dose–effect curves in females vs. males were significantly different, $t(51) = 2.18$, $p = 0.04$. In contrast, bremazocine produced very similar dose-dependent decreases in response rate in both sexes (bottom left panel).

The mixed κ/μ agonist ethylketazocine (Fig. 3, middle left panels) completely substituted for U69,593 in all seven males tested, and in five of seven females tested. After testing all doses of EKC (0.0003–0.3 mg/kg), both females in which no dose of EKC substituted for U69,593 were retested with one or more doses of EKC, and no substitution was obtained upon retest. The highest dose of ethylketazocine tested, 0.3 mg/kg (not shown), eliminated responding in nearly all rats. ED_{50} values in females vs. males were not significantly different: 0.024 (C.L. 0.007, 0.076) vs. 0.014 (C.L. 0.007, 0.028) mg/kg, respectively. Ethylketazocine produced dose-dependent decreases in response rate with no sex differences.

The vehicle used for morphine and BW373U86 (saline) did not produce substantial responding on the U69,593-appropriate lever in rats of either sex (upper middle right panel). The μ agonist morphine (Fig. 3, middle right panel) and the nonpeptidic δ agonist BW373U86 (Fig. 3, right panel) did not substitute for U69,593 in a dose-dependent manner in either sex, although complete substitution occurred at one or more doses of morphine in two females and two males, and at one or more doses of BW373U86 in three females and two males. Both agonists produced dose-dependent decreases in response rate with no sex differences.

Antinociceptive Effect of U69,593

Figure 4 shows the effects of U69,593 on hot-plate antinociception in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. Response latencies after vehicle injection were not statistically different in females and males. U69,593 increased response latency in a dose-dependent manner [dose: $F(3, 36) = 16.71$, $p < 0.001$], with no sex differences.

Diuretic Effect of U69,593

Figure 5 shows the diuretic effect of U69,593 in rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. During the 2 h following a vehicle (10% EtOH) injection, female and male rats urinated approximately the same amount; similar urine output was obtained after saline injection (0.52 ± 0.15 vs. 0.55 ± 0.19 ml for females vs. males, respectively). U69,593 increased urine output in both sexes in a dose-dependent manner, but to a significantly lesser extent in females compared to males [sex × dose: $F(3, 36) = 8.01$, $p < 0.001$]. Because female and male rats differed substantially in body weight, sex differences in urine output were also examined as a function of body weight; statistical analysis of ml urine/kg body weight data showed that U69,593 still produced significantly less effect in females than in males [sex × dose: $F(3, 36) = 4.98$, $p = 0.005$].

Redetermination of U69,593 and Bremazocine Discrimination Curves

The U69,593 discrimination curve was redetermined after all other tests were completed (7–10 months after the first

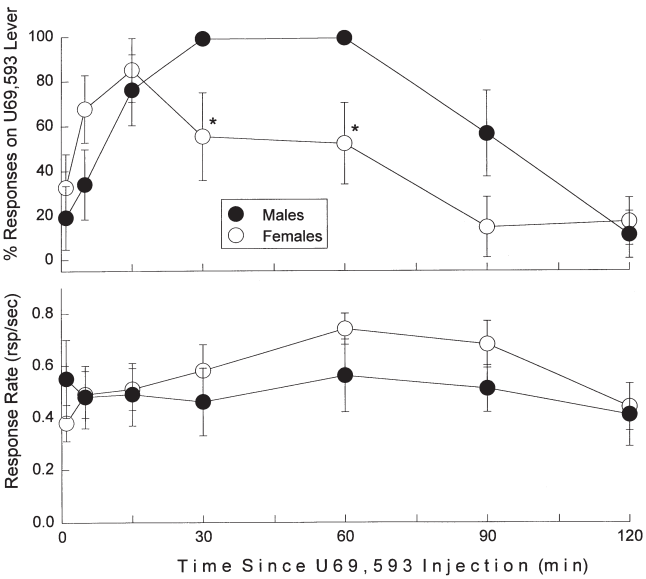

FIG. 2. Time course of discriminative stimulus (top panel) and response rate-altering (bottom panel) effects of U69,593 in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. The training dose of U69,593 was administered, and 1, 5, 15, 30, 60, or 120 min later rats were tested for 5 min only. Each point is the mean ± 1 SEM of seven females or seven males. *Females significantly different from males, $p \leq 0.05$, Tukey test.

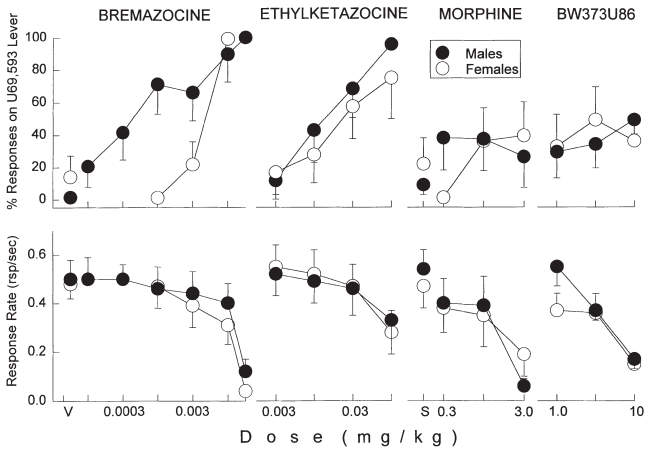

FIG. 3. Discriminative stimulus (top panel) and response rate-altering (bottom panel) effects of vehicle (V), saline (S), κ (bremazocine, ethylketazocine), μ (morphine), and δ (BW373U86) opioid agonists in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. Injections were given 30 min (V, bremazocine), 15 min (ethylketazocine, BW373U86), and 20 min (S, morphine) before the session. Each point is the mean ± 1 SEM of seven females or seven males (bremazocine, ethylketazocine), or six females or six males (morphine, BW373U86).

U69,593 dose–effect determination). The initial sex difference in potency of U69,593 as a discriminative stimulus was diminished: ED_{50} values were 0.065 (C.L. 0.043, 0.106) vs. 0.041 (C.L. 0.031, 0.055) mg/kg for the eight females and eight males, respectively. Only one female still showed any proestrus days during 1 week of sampling at this point in the study, as indicated by vaginal cytology; other females showed only estrus and/or diestrus. Because the U69,593 dose–effect curves had shifted significantly in some rats, bremazocine dose–effect curves then were reexamined in most rats (7–10 months after the first bremazocine dose–effect determination). Up to doses that eliminated responding, bremazocine did not substitute for U69,593 in one of seven females tested, or in two of six males tested. There was no sex difference in potency of bremazocine to substitute for U69,593 at the end of the study: ED_{50} values were 0.0020 (C.L. 0.0009, 0.0032) vs. 0.0015 (C.L. 0.0004, 0.0047) mg/kg in females vs. males, respectively.

Brain/Blood Ratio of [^{3}H]U69,593

Brain/blood ratios of [^{3}H]U69,593 were determined in a separate group of male and female rats that were approximately the same age as the discrimination rats were at the beginning of the experiment. Table 1 shows that there were no sex differences in brain/blood ratio of [^{3}H]U69,593 examined 30, 60, or 90 min postinjection, the time points at which the greatest sex differences were observed in the U69,593 discrimination.

DISCUSSION

The present study showed that there are sex differences in U69,593's initial discriminative stimulus and diuretic effects, but not in its antinociceptive and rate-decreasing effects in the rat. Females took significantly more sessions than males to meet training criteria used for establishing the discrimination. In addition, the initial mean ED_{50} values for U69,593 discrimination and bremazocine substitution were significantly higher in females compared to males, ethylketazocine substituted for U69,593 in slightly fewer females than males, and the time course of U69,593 discrimination differed between the sexes. U69,593 also produced significantly less diuresis/dose in females than in males. In contrast, neither a μ or δ opioid agonist substituted for U69,593 in a majority of rats of either sex, and there were no sex differences in the effects of any opioid agonist on rate of responding in the discrimination task. Moreover, there were no sex differences in magnitude of U69,593-induced hot-plate antinociception, or in brain/blood ratios of [^{3}H]U69,593 measured 30, 60, or 90 min postinjection in a separate group of naive rats. Taken together, these results suggest that sex differences observed in U69,593's discriminative and diuretic effects probably are not due to sex differences in U69,593 pharmacokinetics. The fact that sex differ-

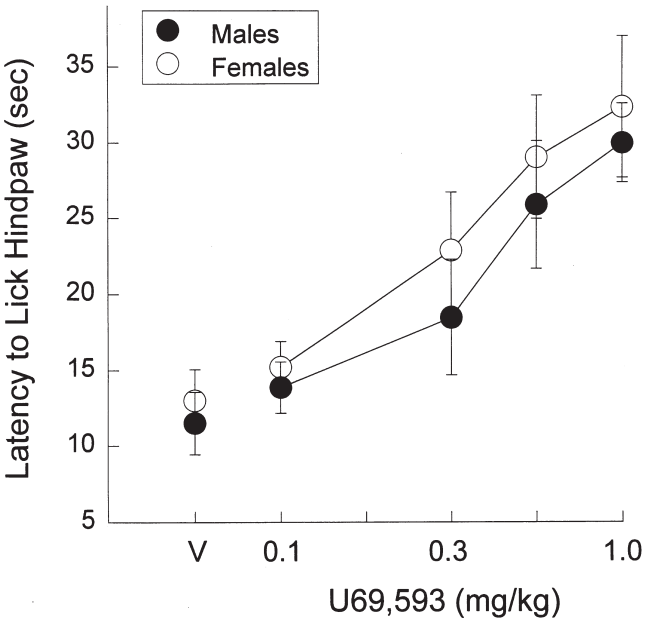

FIG. 4. Antinociceptive effect of U69,593 in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. Vehicle (V) or U69,593 was administered, and 30 min later rats were placed on a 52°C hot plate. Each point is the mean ± 1 SEM of seven females or seven males.

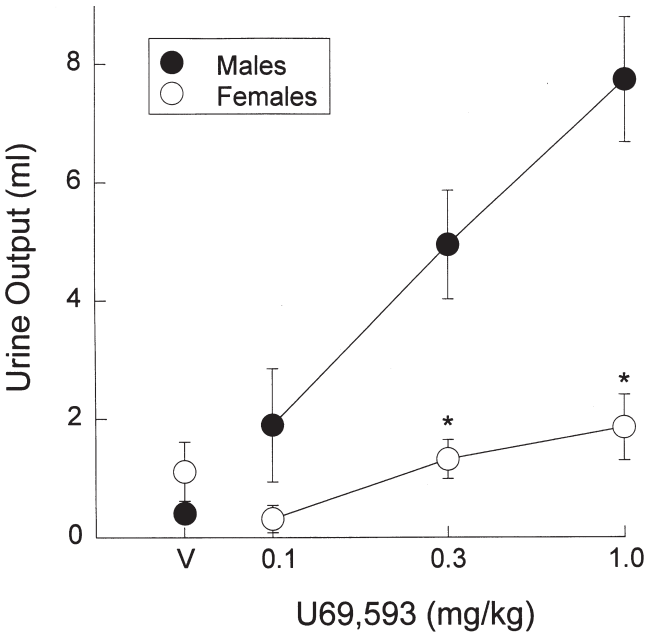

FIG. 5. Diuretic effect of U69,593 in female and male rats trained to discriminate 0.13 mg/kg U69,593 from vehicle. Vehicle (V) or U69,593 was administered, and rats were immediately placed into a chamber for 2 h. Each point is the mean ± 1 SEM of seven females or secen males. *Females significantly different from males, $p \leq 0.05$, Tukey test.

TABLE 1

BRAIN/BLOOD RATIOS* (MEAN ± 1 S.E. OF [^{3}H]U69,593 IN MALE VS. FEMALE RATS†

Time postinjection:	30 min	60 min	90 min
Males	4.05 ± 0.32	3.77 ± 0.96	3.25 ± 0.32
Females	3.81 ± 0.48	3.33 ± 0.90	4.03 ± 0.37

*cpm/g brain ÷ cpm/ml blood.
†n = 4/sex/time point.

ences in potency of U69,593 and bremazocine as discriminative stimuli diminished substantially over the course of the study suggests that factors that changed over time, such as additional discrimination training, repeated drug administration, age, and hormonal status, were important determinants of the initial sex difference in discriminability of U69,593.

There are no previous discrimination studies in which U69,593 has been used as the training drug. However, the general pattern of acquisition and substitution results obtained in the present study agrees with that of previous studies using other κ opioids as training drugs, in male rats. In the present study, only two rats—both males—acquired the initial 0.1 mg/kg U69,593 discrimination within the first several weeks of training; including sessions conducted after the training dose was increased to 0.13 mg/kg, sessions to an accurate discrimination averaged 44 in male rats. Mean number of sessions to meet training criteria using a closely related compound, U50,488, was approximately 45 sessions in male rats [5.6 mg/kg (35)], whereas it took 25–45 sessions in male rats trained to discriminate bremazocine from vehicle (41,44), and approximately 30 sessions in male rats trained to discriminate spiradoline (20) or ethylketazocine (42) from vehicle. In contrast, female rats in the present study required significantly longer training time than males did (an average of 67 sessions, which does not include one female that did not meet training criteria within 105 sessions). This appears to be beyond the average previously reported for male rats trained to discriminate other kappa agonists from vehicle, although comparisons across training drugs are limited by the difficulty of equating training doses across drugs.

U69,593 also was significantly less potent in producing discriminative effects in females compared to males (ED_{50} 0.074 vs. 0.025 mg/kg, respectively), initially. Individual differences in potency of the training drug have been reported previously for apomorphine (40) and Δ^9-tetrahydrocannabinol (30) in male rats, in which higher ED_{50} values for discrimination were correlated with slower acquisition, as in the present study. U69,593 also had an earlier peak effect (15 vs. 30 min) and shorter duration of action in females compared to males. Two previous reports on the time course of U50,488 discrimination indicate a substantially longer time course than that found in the present study, although peak effects also occurred at 30 min postinjection in those studies (3.0–4.2 mg/kg U50,488 discrimination (35,36)). Given that the pretreatment time during training was 30 min in the present study, it is particularly surprising that females peaked at 15 min postinjection. In fact, this result suggests that if a 15-min pretreatment time had been used, females may have acquired the discrimination at a rate equal to that of males, with no difference in substitution ED_{50}. However, this shorter pretreatment time was used initially (with the 0.1 mg/kg training dose), yet no females acquired the discrimination within 6 weeks, whereas two of six males did. Thus, it is more likely that training female rats at both a higher dose of U69,593 and a shorter pretreatment time would be necessary to eliminate the sex differences in acquisition.

Although U69,593 dose–effect curves in males and females were separated by approximately 1/4 log unit at the beginning of the study, the sex difference in potency was no longer statistically significant at the end of the study. Furthermore, the initial sex difference in bremazocine's potency was also no longer observed upon retesting 7–10 months after the first dose–effect curve was obtained. We previously reported similar instability over time of an initial sex difference in discriminative stimulus effects of morphine (11); whether these small changes are due to aging, hormonal fluctuations, learning, or some other variable is not known. It is possible, for example, that initial sex differences were due to insufficient discrimination training, so that asymptotic performance simply took longer in females than in males. Additionally, a number of factors that change with age, such as hormonal status, could influence discriminability of U69,593 over the course of a long-term study. Whereas all but one female showed evidence of regular estrous cycling during the time the first U69,593 discrimination curve was obtained (when females were approximately 6–7 months old), almost no females appeared to be cycling regularly at the time the second curve was obtained (when females were approximately 13–19 months old). Selective manipulation of hormonal status in young males and females would clarify whether this is an important factor in discriminability of U69,593, although we have not found gonadal hormones to substantially influence discriminability of morphine (9).

Patterns of agonist substitution in the present study generally agree with those of previous studies in male animals, albeit using different agonists as training drugs. For example, the κ agonist bremazocine, which completely substituted for U69,593 in nearly all rats in the present study, has been shown to completely substitute for U50,488 in male rats [3.0–5.6 mg/kg U50,488 (32,35)] and male monkeys [0.75–1.7 mg/kg U50,488 (33)]. Similar to U69,593, however, bremazocine was significantly less potent in females than in males in producing U69,593-like discriminative effects. Additionally, the slope of the bremazocine dose–effect curve was significantly different in females vs. males, suggesting possible qualitative differences in the U69,593 interoceptive cue between females and males. Another agonist tested, ethylketazocine, completely substituted for U69,593 in all males and in five of seven females; however, unlike U69,593 and bremazocine, the mean ED_{50} for ethylketazocine substitution was only slightly higher in females compared to males (0.024 vs. 0.014 mg/kg, respectively). Previous studies indicate that ethylketazocine completely substituted for U50,488 or bremazocine in all male rats discriminating either a low or high dose of these kappa agonists (35,41,44). Thus, it is not clear whether the failure of ethylketazocine to substitute for U69,593 in two female rats represents a meaningful sex difference. In addition to its κ agonist activity, ethylketazocine possesses μ agonist activity in male rodents in some procedures [e.g., antinociception (14,38) and drug discrimination (29)], but not others [schedule-controlled responding (37,45)]. We previously reported that females were more sensitive than males to morphine's discriminative stimulus effects (lower ED_{50} for morphine in females than in males) (11); perhaps females in the present study were more sensitive than males to the μ component of ethylketazocine's effects. However, this hypothesis would also predict that the more μ-selective agonist morphine would substitute for U69,593 to a lesser extent in females than in males, which did not occur. An alternative explanation for lack of sex differences in ethylketazocine's potency is that it was tested several months after U69,593 and bremazocine; sex differences in potency of U69,593 and bremazocine diminished from the beginning to end of the study, suggesting that the likelihood of observing sex differences in the potency of any κ agonist decreased over time.

The failure of a μ or a δ opioid agonist to substitute for U69,593 in most female and male rats in the present study agrees with previous studies of opioid discriminations using different κ agonists as training drugs. The δ agonist BW373U86 did not substitute for U69,593 in a dose-dependent manner in

either sex in this study, or in previous studies of bremazocine discrimination in the pigeon (8,31). The μ agonist morphine also did not substitute for U69,593 in a dose-dependent manner in either sex in the present study; similarly, morphine has not substituted for U50,488 (32,35) or bremazocine (41) in previous studies using male rats. Considering all κ, μ, and δ agonist substitution tests together, the fact that there were only a few sex differences in maximal substitution or slope of the substitution curve suggests that the U69,593 discriminative stimulus is qualitatively similar in male and female rats. However, this conclusion can only be confirmed by broadening the range of opioid agonists examined in substitution tests and conducting antagonism studies to clarify the nature of the sex differences observed, especially with bremazocine and ethylketazocine.

In addition to being less potent as a discriminative stimulus, U69,593 was also considerably less potent as a diuretic in females than in males, even when sex differences in body weight were taken into account. It is unlikely that this sex difference is due to different hydration levels in females and males, because there was no sex difference in urine output under saline control conditions. The dramatic sex difference in slope of the diuresis dose–effect curves indicates that U69,593 also may be less efficacious as a diuretic in females than in males, although this can only be confirmed by examining the effects of higher doses of U69,593. Previous studies have documented the marked diuretic effects of κ agonists, including U69,593, in male rats (27,28,46); in fact, opioid-induced diuresis is considered a marker for κ receptor-mediated activity (25,26). Sex differences in κ agonist-induced diuresis could reflect sex differences in the activity of vasopressin, a neuroactive peptide that has a major role in excretion of water from the body. One mechanism by which κ agonists produce diuresis is suppressing central vasopressin release (27,28,39,50), and there are significant sex differences in vasopressin systems in the brain—with males showing considerably more activity than females (12,13,49). Thus, it is likely that U69,593-induced diuresis was greater in males than in females because, at least in part, vasopressin activity is greater in males than in females. This hypothesis could be tested by measuring vasopressin levels in vehicle- vs. U69,593-treated male and female rats. In addition, it will be important to determine the generality of sex differences in κ agonist-induced diuresis, including a comparison to standard diuretics that act via different mechanisms.

Although U69,593 was less potent in females than males in its initial discriminative and diuretic effects, there were no sex differences in the antinociceptive effects of U69,593 on the hot-plate assay. Currently, there is no clear consensus on sex differences in κ agonist-induced antinociception (2,17,18,21), although the present results agree with our previous finding of no sex difference in potency (or time course) of U69,593-induced hot-plate antinociception in rats (2).

The fact that sex differences in U69,593's effects were inconsistent across behavioral and physiological end points examined in the same rats, and no sex differences in brain/blood ratios of [^{3}H]U69,593 were observed in a separate group of rats, suggests that the mechanism underlying the observed sex differences is probably not a pharmacokinetic one. A hypothesis of differential pharmacokinetics would predict that U69,593 would be more potent in females than in males for all centrally mediated effects of U69,593, yet sex differences in potency of U69,593 were not consistent among all behavioral effects measured. Additionally, a pharmacokinetic hypothesis would predict that brain/blood ratios of [^{3}H]U69,593 would be higher in males than in females at 30–90 min postinjection, a time period in which U69,593 was a more potent discriminative stimulus and diuretic in males than in females. We are not aware of any previous studies examining sex differences in pharmacokinetics of U69,593, or any other selective κ agonist. Additionally, there is little evidence for sex differences in opioid pharmacodynamics, such as receptor (and/or dynorphin) density and distribution; however, almost no investigators have addressed this question in structures other than hypothalamic nuclei (19,43). The fact that sex differences in U69,593's effects were not consistent across all physiological and behavioral end points, and there were no sex differences in a preliminary pharmacokinetic analysis, indicates that pharmacodynamic differences must be considered.

In conclusion, the present study indicates that there are sex differences in the initial discriminative stimulus and diuretic—but not response rate-decreasing and antinociceptive—effects of U69,593 in rats. The lack of consistency of sex differences across behavioral and physiological end points, and the lack of a sex difference in brain/blood ratio of [^{3}H]U69,593 indicates that sex differences in pharmacodynamics may underlie the observed differences in behavioral and physiological effects of U69,593 in female vs. male rats. Furthermore, factors that changed over time, such as additional training, age, and hormonal status appeared to be important determinants of initial sex differences in discriminability of U69,593.

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