Sex Differences in the Pentylenetetrazol-Like Stimulus Induced by Ethanol Withdrawal

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ABSTRACT
This study investigated sex differences in responding to the pentylenetetrazol (PTZ, a $\gamma$-aminobutyric acid A antagonist) discriminative stimulus and to substitution to PTZ during ethanol withdrawal. The PTZ stimulus has served as an anxiogenic stimulus in numerous studies. Adult male and female rats were trained to discriminate PTZ (16 mg/kg i.p.) from saline in a two-lever food-reinforced task. They were then gonadectomized or sham-operated. Ovariectomized (OVX) rats were also tested during 17$\beta$-estradiol (2.5 mg, 21 days release, s.c.) replacement. The PTZ dose response (0–16 mg/kg i.p.) was tested in all groups. In general, fewer females than males responded to PTZ. Diazepam (DZP; 0–10 mg/kg i.p.) injected before PTZ (16 mg/kg) decreased the number of rats selecting the PTZ lever. This effect was greater in sham female and estradiol-replaced-OVX rats than in male or OVX rats. Rats then received chronic ethanol diet (6.5%) for 10 days. During ethanol withdrawal (12 h after termination of the ethanol diet), they were tested for PTZ lever selection. PTZ lever selection differed between groups: sham or castrated male rats > OVX > sham female or estradiol-replaced-OVX rats. In sham female rats, estradiol concentrations showed a cyclic pattern with an estradiol surge that did not influence their PTZ discrimination performance. After i.p. injection of ethanol (2 g/kg), blood ethanol concentrations were not different in male and female rats. These findings suggest that 1) female rats are less sensitive to the anxiogenic effects of PTZ; 2) female rats are less sensitive to the anxiogenic effects of ethanol withdrawal; and 3) estrogen plays some role in mediation of these sex differences.

Anxiety is one of the major symptoms of ethanol withdrawal (EW), and avoidance of EW-induced anxiety is a critical factor in the maintenance of ethanol consumption in alcoholics. However, most ethanol studies have focused on male subjects despite the fact that one-third of alcoholics in the United States are women and despite the evidence that men and women differ in their physiological and behavioral response to ethanol (Rivier, 1993; Blanchard and Glick, 1995).

Moreover, the subjective nature of anxiety makes it difficult to assess in both men and women. Pentylenetetrazol (PTZ), at subconvulsant doses, is a prototype anxiogenic drug in humans and the PTZ discrimination assay has been extensively used as an animal model of anxiety (Lal and Fielding, 1979; Lal et al., 1981; Lal and Emmett-Oglesby, 1983). Benzodiazepines (Shearman and Lal, 1979; Gherezghiher and Lal, 1982) block the discriminative effects of PTZ, whereas $\gamma$-aminobutyric acid (GABA)A antagonist drugs potentiate it (Shearman and Lal, 1979; Idemudia et al., 1989). These findings reinforce the anxiogenic nature of the PTZ discriminative stimulus, and indicate a GABA A-related mechanism. Furthermore, rats trained to discriminate PTZ select the drug lever during EW, and this effect is potentiated by GABAA antagonists and reversed by benzodiazepines (Lal et al., 1988; Idemudia et al., 1989; Prather et al., 1992). Taken together, these findings suggest that PTZ drug discrimination is a good model for EW-induced anxiety and that GABA A receptors are largely involved.

Sex differences in behavioral responses to anxiogenic stimuli have been reported in numerous studies with conflicting results depending on anxiety models used. Female rats show a lower level of anxiogenic behavior than male rats in an elevated plus-maze test (Johnston and File, 1991) or in an inescapable foot-shock test (Heinsbroek et al., 1990). Fewer female than male rats show a shock stress-induced decrease in locomotor activity (Heinsbroek et al., 1990). In contrast, female rats show a greater anxiogenic response than male rats in a social interaction test and a conflict test (Johnston and File, 1991), and female rats release more corticosterone after swim stress (Wilson and Biscardi, 1994). Because anxiety is a complex central nervous system disorder with numerous hormonal and neuronal factors involved, the sensitivities of men and women differ.

ABBRVIATIONS: EW, ethanol withdrawal; PTZ, pentylenetetrazol; BEC, blood ethanol concentration; DZP, diazepam; FR, fixed-ratio; GABA, $\gamma$-aminobutyric acid; OVX, ovariectomy.
trogen concentration and PTZ-lever responding. Estradiol replacement, to determine the relationship between es-
sured in sham female and OVX rats with and without estra-
male rats. Furthermore, estradiol concentrations were mea-
diol-replaced OVX rats will respond more like female rats
nist such as DZP than will male rats; 3) OVX rats will
have been found in higher levels in the brain of female rats
respond to the PTZ discriminative stimuli or the
withdrawal induced anxiety; 2) as reviewed above, the effects
above, PTZ has been shown to be a useful model of ethanol-
differences in the anxiety-like behaviors measured by the
PTZ drug discrimination assay between male and female
rats. PTZ is useful for a number of reasons: 1) as described
above, PTZ has been shown to be a useful model of ethanol-
threshold for the PTZ-induced seizure is higher in female
rats than in male rats, and this difference is abolished by
ovariectomy (Kokka et al., 1992). GABA-mediated chloride
conduction in the GABA_A receptors is higher in intact fe-
male rats than in ovariecimized (OVX) rats (Bitran et al.,
1991). Progesterone, a major female steroid, and its neuro-
active metabolites are thought to enhance a GABA_A agonistic
activity (Majewski, 1991). These GABA_A agonistic steroids
have been found in higher levels in the brain of female rats
than in male rats (Corpechot et al., 1993).

To this end, we tested the hypotheses that: 1) fewer female
rats will respond to the PTZ discriminative stimuli or the
PTZ-like stimulus induced by EW than will male rats; 2) more female rats will respond to a benzodiazepine site ago-
nist such as DZP than will male rats; 3) OVX rats will
respond more like male than like female rats; and 4) estra-
diol-replaced OVX rats will respond more like female rats
than the OVX rats. In addition, an analysis of blood ethanol
concentrations (BEC) was conducted in this study to exclude
the possibility that a lower BEC in female rats during etha-
nol exposure results in lower EW-induced anxiety than in
male rats. Furthermore, estradiol concentrations were mea-
ured in sham female and OVX rats with and without estradi-
ol replacement, to determine the relationship between es-
rogen concentration and PTZ-lever responding.

Materials and Methods

Animals. Adult male and female Long-Evans hooded rats
(Charles River, Wilmington, MA) were housed individually with
temperature (22–25°C) and humidity (55%) held constant. A 12-h
light/dark cycle was maintained with lights on between 7 AM and 7
PM. Animal body weights were maintained at 320 to 350 g for male
rats and 290 to 310 g for female rats by limiting food (Purina rat
chow) to 20 g/day for males and 16 g/day for females, including the
food received during training. Water was available ad libitum.

Discrimination Training. Gonadally intact male and female
rats were trained to press a lever for food reward under a fixed-ratio
1 (FR1) followed by a FR3 schedule. This procedure required animals
to learn a lever-press response. After acquisition of a lever-press
response, they were trained to discriminate between PTZ (16 mg/kg
i.p.) and saline under a FR10 schedule. No seizure activity of any
kind was observed after the training dose of PTZ. One-half of the
male and female rats were trained with PTZ as the cue on the right
lever, and one-half were trained with PTZ on the left lever. During
each session, the rats received an injection of either saline or PTZ.
Fifteen minutes later, the rats were placed in an operant chamber.
Each training session lasted for a maximum of 10 min, and the rats
could earn up to 24 food pellets. An equal number of saline and PTZ
training sessions was given in an irregular order of presentation
such that no condition occurred in more than three consecutive
tests. Animals received approximately 60 training sessions in total before
use in any behavioral experiment. Animals were selected for use in
experiments when they achieved a 90% correct response rate for their
last 10 training sessions.

Discrimination Testing. The percentage of animals selecting the
PTZ lever was measured after treatment with increasing doses of
PTZ (0, 4, 8, 16 mg/kg i.p.). A cumulative dosing regimen was applied
in the following manner. Fifteen minutes after injection with saline,
animals were tested for 2 min to determine their lever selection. The
test session ended with the delivery of a single food pellet on com-
pletion of FR10 or, if neither lever was selected, after 2 min had
elapsed. On completion of the lever selection, animals were immedi-
ately injected with PTZ 4 mg/kg and tested as above. The next dose
injected was 4 mg/kg PTZ, which was the difference (4 mg/kg),
between the second PTZ dose (8 mg/kg) and the first PTZ dose (4
mg/kg). In this manner, animals were given additional doses and
tested until they received a cumulative dose of PTZ (16 mg/kg).

Chronic Ethanol Administration and EW Tests. Ethanol was
administered in a nutritionally complete liquid diet containing 6.5%
ethanol (w/v) as modified by Dodd and Shorey-Kutschke (1987).
Animals received 100 ml of the ethanol diet each morning for 9 days.
On the morning of the 10th day, 50 ml of the diet was given. Twelve
hours later, the diet tubes were removed and animals were given free
access to food. In the morning of the first day after termination of the
chronic diet, rats were injected with saline and were examined for
their PTZ-lever selection.

Gonadectomy. This procedure was applied to male and female
rats that had acquired the PTZ discrimination task. Male (30) and
female (30) rats were assigned into a sham-operated (N = 15 for each
sex) or a gonadectomized (N = 15 for each sex) group. In a separate
experiment (experiment 1), a separate group of PTZ-naive female
rats (15) were ovariecimized before the PTZ discrimination train-
ing. Ovariectomy was performed under ether anesthesia. A small
incision was made in the abdominal cavity directly above the ovaries.
The ovaries of the OVX group were removed bilaterally, whereas
those of the sham group were left intact. The incisions were closed
with stainless steel wound clips. For castration, a small incision was
made in the scrotum. After removal of the testes, two sutures were
used to close the incision. Animals were returned to the colony room
and were allowed a 2-week recovery period before initiating behav-
ioral anxiety tests.

β-Estradiol Replacement. Preliminary results indicated that
castrated male rats do not significantly differ from sham-operated
male rats in the PTZ discrimination stimulus. Thus, hormone re-
placement was conducted only for OVX rats that had already ac-
quired the PTZ discrimination task. Twelve OVX rats were s.c.
implanted with β-estradiol pellets (2.5 mg for 21 days release). After
a 2-day recovery period, they were subjected to behavioral tests.

Blood Ethanol Analysis. Subjects for this analysis were gonad-
ally intact male (5) and female (5) rats. All rats were first aneste-
thized with a combination of ketamine (100 mg/kg) and chlorodiap-
oxide (20 mg/kg) dissolved in saline. Thereafter, a catheter was
inserted into the right external jugular and the free end was fixed to
the skull. After a 5-day recovery period, they were injected with
ethanol (2 g/kg, 20% i.p.). Whole blood samples (100 µl) were taken
from each rat through the jugular catheter at five different time
dates.
Blood β-Estradiol Analysis. Three groups of female rats were used for this assay: sham-operated (five), ovariectomized (five), and β-estradiol replaced ovariectomized (five). Whole blood samples (0.75 ml) were taken from each rat by the same method described above.

For sham female rats, blood was collected for 5 consecutive days (days 1–5), whereas for OVX and β-estradiol-replaced OVX rats, blood was taken on days 1 and 5 corresponding to sham female rats. Blood samples were immediately centrifuged and at least 250 μl of serum was collected for assay. Each serum sample was kept frozen until assayed for β-estradiol concentration by radioimmunoassay. Serum samples were incubated with 125I-estradiol in antibody-coated tubes for 3 h at room temperature. 125I-Estradiol competes with estradiol in the sample for antibody sites. After incubation, separation of "bound" from "free" was achieved by decanting. With the use of a foam decanting rack, the contents of all tubes were aspirated, and the radioactivity was counted using a gamma counter. The quantity of estradiol in the sample was determined by comparing the counts to a calibration curve.

Drugs. PTZ was purchased from Sigma (St. Louis, MO). PTZ was freshly prepared and was dissolved in the saline solution (0.9%). DZP was kindly donated by Hoffmann-La Roche (Nutley, NJ) and was homogenized in 3% carboxymethyl/cellulose solution. β-Estradiol (2.5 mg/pellet, 21-day release) was purchased from Innovative Research of America (Sarasota, FL).

Experimental Procedure. The number of rats used for behavioral tests varied depending on the number of rats that met the test criterion at the time of testing. In addition, during testing, animals that failed to emit a lever-press response (one FR10) within a given test time (2 min) were not used in data analysis. For behavioral experiments, five experimental (sex) groups were tested: 1) sham-operated males; 2) castrated males; 3) sham-operated females; 4) OVX females; and 5) estradiol-replaced OVX females. For experiment 1, a separate group of OVX rats ovariectomized before training was added. Rats that met the criterion for the PTZ discrimination task by pressing the correct lever in 9 of 10 consecutive training sessions were selected in behavioral tests.

Experiment 1 was designed to determine whether the five experimental groups differ in their dose response to a GABAA antagonist, PTZ (0, 4, 8, and 16 mg/kg i.p.), in the PTZ discrimination task. Sham-operated male (12) and female (12) rats, gonadectomized male (12) and female (12) rats, and estradiol-replaced OVX rats (11) were injected with saline and PTZ in a cumulative dosing method. They were then tested for their PTZ-lever selection at each dose of PTZ.

In addition, the PTZ dose response was compared between female rats trained with and without ovaries to determine possible differences in stimulus properties or intensity of the PTZ discriminative stimulus.

Experiment 2 was designed to determine whether the five experimental groups differ in their dose response to a benzodiazepine site agonist, DZP (0, 0.625, 1.25, 2.5, 5.0, or 10 mg/kg i.p.). Sham-operated male (15) and female (11) rats, gonadectomized male (13) and female (13) rats, and estradiol-replaced OVX rats (11) were injected with CM-cellulose (3%, vehicle) or one dose of DZP 15 min before PTZ injection. Fifteen minutes after PTZ, the rats were tested for PTZ-lever selection. Each dose of DZP in combination with PTZ (16 mg/kg) was tested on a different day in a randomized order of DZP doses with a recovery period (3–7 days) between tests. The length was decided based on the animals’ PTZ discriminative performance during the period. Thus, when 90% of animals respond to a correct lever after PTZ (16 mg/kg) or saline, they were tested for DZP on the next day.

Experiment 3 was designed to determine whether the five experimental groups differ in development of an endogenous PTZ-like stimulus during acute EW (12 h after termination of ethanol diet). Sham-operated male (12) and female (11) rats, gonadectomized male (12) and female (12) rats, and estradiol-replaced OVX rats (12) received ethanol diet for 10 days. Twelve hours after termination of the ethanol diet, they were given a saline injection and tested for PTZ-lever selection.

Experiment 4 was designed to determine whether the five experimental groups differ in their dose response to the PTZ discrimination stimulus during protracted EW (36 h after termination of ethanol diet). Sham-operated male (12) and female (11) rats, gonadectomized male (12) and female (12) rats, and estradiol-replaced OVX rats (12) received ethanol diet for 10 days. Thirty-six hours after removal of ethanol diet, they were given saline and PTZ 4, 8, and 16 mg/kg (cumulative doses). Fifteen minutes later, they were tested for PTZ-lever selection.

A control experiment was designed to determine whether gonadally intact male and female rats differ in the BEC and clearance rate for blood ethanol before ethanol exposure and during EW. Gonadally intact male (5) and female (5) rats were injected with 2 g/kg ethanol and blood samples (100 μl) were collected at five time points, 0 (before ethanol injection), 15, 30, 60, and 120 min after ethanol injection. Blood samples were then refrigerated until assayed. Animals then received chronic ethanol diet for 10 days. Twelve hours after termination of the ethanol diet, rats were injected with 2 g/kg ethanol. Blood samples (100 μl) were collected in the same manner as described above. Ethanol concentration was analyzed by head space gas chromatography.

A second control experiment was designed to determine whether a relationship exists between estrogen concentration and the occurrence of the PTZ-induced discriminative stimulus in sham female and OVX rats. β-Estradiol concentration was measured in sham female rats (five) for 5 consecutive days (days 1–5). In OVX rats (five) and β-estradiol-replaced OVX rats (five), the concentration of β-estradiol was measured on corresponding days 1 and 5. The same sham female rats (five) were then used for the PTZ-lever selection after PTZ (16 mg/kg) and saline injection.

Data Analysis. The data for selection of the PTZ lever were expressed as percentages, which were obtained as follows: % = 100 × (no. of rats selecting the PTZ-lever/total no. of rats that completed the test). For calculation of ED50 values, the PTZ dose-response data were plotted as a logit PTZ-lever selection versus a log dose of PTZ. Each test session (a dose-response test, PTZ-lever selection tests during acute or protracted EW) was conducted three times to obtain the mean and S.E. For the purposes of analysis, experimental groups defined by the variable "sex" included sex, surgical condition, and hormonal treatment (five groups).

Experiments were analyzed by appropriate repeated-measures ANOVA. A priori repeated-measures contrasts were conducted to compare effects of each group when two-way ANOVA was used. If a significant effect was seen, post hoc tests (Bonferroni) were conducted between individual means. For experiments 1 and 2, data were analyzed by two-way ANOVA (sex × dose). In experiment 3, a one-way ANOVA (sex) was used. In experiment 4, the sex difference in ED50 was analyzed by two-way ANOVA (sex × ethanol condition). For control experiments, data were analyzed by one-way ANOVA by sex (BEC) or day (estradiol concentration). For determination of estradiol concentrations in sham female rats, data were collected for a 4-day cycle of 5 days. A peak estradiol concentration was synchronized on day 2 because two of five rats had the peak on day 2. The significance level was set a priori at P < .05.

Results

After approximately 60 training sessions, animals acquired the PTZ discrimination task; they selected a PTZ lever after PTZ injection (16 mg/kg i.p.) and a saline lever after saline injection.
Experiment 1: Sex Difference in the Dose-Response Effect of the PTZ Discrimination. As the dose of PTZ increased, PTZ-lever selection (Fig. 1) also increased in all groups of rats with dose and sex group interaction \((F_{12,30} = 22.7, P < .001)\). Five experimental groups differed in the PTZ dose response \((F_{4,10} = 44.5, P < .001)\). A repeated-measures analysis by dose was conducted for each pair of experimental groups. PTZ-lever selection (percentage) was lower in the sham female group than in the sham male group \((F_{1,4} = 108.9, P < .001)\) or in the OVX group \((F_{1,4} = 25.9, P < .007)\). PTZ-lever selection (percent) was also lower in the estradiol-replaced OVX group than the OVX group \((F_{1,4} = 12.9, P < .023)\). No significant differences were observed between two male groups (sham and castrated) and between the sham female and the estradiol-replaced OVX groups. In addition, the dose-response effect of female rats ovariectomized before the PTZ discrimination training did not differ from that of female rats ovariectomized after the PTZ discrimination training.

The percentage of rats selecting the PTZ-lever was lower in the sham female group or the estradiol-replaced OVX group than in the sham male, castrated male, or sham female groups \((P < .05)\). At 8 mg/kg PTZ, the percentage of rats selecting the PTZ lever was lower in all the females groups than in the male groups \((P < .05)\) at 16 mg/kg, no differences between groups was observed.

Experiment 2: Sex Difference in the Effect of a Benzodiazepine Site Agonist, DZP on the PTZ Discrimination Stimulus. DZP \((0, 0.625, 1.25, 2.5, 5.0,\) and 10 mg/kg i.p.) injected before PTZ administration \((16\) mg/kg i.p.) blocked the PTZ discrimination stimulus (Fig. 2). This effect of DZP was greater as dose increased \((F_{4,52} = 74.9, P < .001)\) and was different in five experimental groups \((F_{4,13} = 28, P < .001)\) with a dose and sex group interaction \((F_{4,16} = 4.6, P < .001)\). The inhibitory effect of DZP was more pronounced in the sham female group than in the sham male \((F_{1,6} = 67.5, P = 0.001)\) or the castrated male group \((F_{1,50} = 80.5, P = .001)\). However, no significant differences were observed between the OVX group and the sham female group or the estradiol-replaced OVX group. Sham female and estradiol-replaced OVX rats responded less on the PTZ lever than did male or castrated male \((P = .05)\) rats at all doses. The OVX rats were different from the male rats only at the 2.5 and 5.0 mg/kg doses \((P = 0.05)\), and were higher than the other female groups at low doses: 0.625 and 1.25 mg/kg.

Experiment 3: Sex Difference in the PTZ-Like Stimulus Induced by Acute EW. During acute EW (Fig. 3), animals injected with saline selected the PTZ lever, and the magnitude of this phenomenon differed in five experimental groups \((F_{4,25} = 6.6, P = 0.001)\). The percentage of rats selecting the PTZ lever was lower in the sham female group \((23.8 \pm 7\%)\) than in the sham male \((50.1 \pm 4\%)\) \((F_{1,16} = 22.7, P < .001)\) or the castrated male group \((42.8 \pm 1.8\%)\) \((F_{1,10} = 5, P = 0.049)\). The percentage in the OVX \((F_{1,13} = 9.4, P = 0.009)\) or estradiol-replaced OVX groups \((F_{1,10} = 13.5, P = 0.004)\) were also lower than that in the sham male group. The OVX rats \((33.5 \pm 4.7\%)\) selected the PTZ lever at a level intermediate between those of the male groups and the sham female or estradiol-replaced OVX rats \((23.8 \pm 6.7\%)\), although this value was not significantly different from those of either the male groups or the female and estradiol-replaced OVX groups. No significant differences were observed between the two male groups (sham and castrated) or between the sham female and the estradiol-replaced OVX groups.

Experiment 4: Sex Difference in the PTZ Discrimination Stimulus during Protracted EW. Figure 4 represents the ED50 values for the PTZ discrimination stimulus before chronic ethanol diet and during protracted EW. Two-way ANOVA revealed a significant difference in the ED50 values by group \((F_{4,20} = 9.9, P < .0001)\) or by ethanol condition \((F_{1,20} = 126.8, P < .0001)\), but no interaction between

**Fig. 1.** Demonstration of a sex difference in the PTZ discriminative stimulus. The abscissa indicates cumulative PTZ doses (mg/kg). The ordinate indicates percentage of rats selecting the PTZ-lever. Data points show the mean values of three determinations, and error bars show the S.E.M.

**Fig. 2.** Demonstration of a sex difference in the effect of DZP on the PTZ discriminative stimulus. The abscissa indicates DZP doses before PTZ (16 mg/kg), and each dose of DZP was tested on a different day. The ordinate indicates percentages of rats selecting the PTZ-lever. Data points show the mean values of three determinations, and error bars show the S.E.M.

**Fig. 3.** Demonstration of a sex difference in PTZ-lever selection during acute EW (AEW). The ordinate indicates percentages of rats selecting the PTZ-lever. Data points show the mean values of three determinations, and error bars show the S.E.M.
and females was about the same under each condition. This indicates that the sex difference is not affected by chronic treatment with ethanol or by withdrawal.

Others have reported that females are less sensitive to EW than males. Devaуд et al. (1995) found that female rats had a lower responsiveness to bicuculline-induced seizure than male rats during EW. In addition, chronic ethanol did not change GABA<sub>A</sub> receptor α1 subunit peptide levels in female rat cortex, but decreased it in male rat cortex (Devaуд et al., 1998).

DZP blocked the discriminative stimulus effects of PTZ. A lack of dose response may result from acute tolerance development to DZP effects: the same rats received multiple DZP injections over a period. Supportive of this view, there was a tolerance development to anxiolytic effects of DZP (Fernandes and File, 1999). Nonetheless, this blockade effect of DZP is in agreement with earlier findings with anxiolytic benzodiazepines such as chloridiazepoxide, flurazepam, and clazobam (Shearman and Lal, 1979; Gherezghiher and Lal, 1982; Jarbe and Hiltunen, 1988). GABA agonists active at the neurosteroid-binding site also block PTZ responding (Beekman et al., 1998). Furthermore, anxiogenic GABA antagonists such as bicuculline and flumazenil increase PTZ lever responding (Shearman and Lal, 1979; Harris et al., 1987). By comparison, non-GABAergic drugs such as d-amphetamine and methylphenidate do not alter the PTZ stimulus (Shearman and Lal, 1979). Similar effects of GABA agonists and antagonists on PTZ-lever responding are seen during EW (Lal et al., 1988; Idemudia et al., 1989; Prather et al., 1992). Taken together, these findings suggest that the GABA<sub>A</sub> receptor plays a major role in the mediation of the anxiety-like discriminative stimulus effects of PTZ.

PTZ also acts at voltage-gated sodium (Brown et al., 1992) and potassium (Sugaya et al., 1989; Madeja et al., 1994, 1996; Klocker et al., 1996) channels, which are thought to mediate its convulsant actions. However, studies of the convulsant effects of PTZ in intact rats typically use doses substantially higher (35–70 mg/kg) (Del Bel et al., 1998; Fischer and Kittner, 1998; Mares, 1998; Dufour et al., 1999) than the 16 mg/kg training dose of PTZ. Furthermore, unpublished data from our laboratory show that whereas other compounds such as calcium channel blockers are capable of modulating PTZ responding, none of them produce the large magnitude shifts in PTZ responding that GABAergic compounds do. These findings, in combination with the fact that no convulsant activity was seen in these rats, suggest that these sites play little role in the anxiogenic or discriminative stimulus effects of PTZ.

Female rats were more sensitive to the effects of DZP on PTZ-lever responding. Given the above-mentioned studies on the importance of the GABA<sub>A</sub> receptor for mediation of PTZ discriminative stimulus effects, this finding provides preliminary evidence that at least part of the differences in PTZ-lever responding in male and female rats may be mediated by GABA<sub>A</sub> receptors.

In the present study, OVX rats produced levels of respond-
ing to the PTZ stimulus intermediate between the male and intact female rats in the experiments described above. Estradiol replacement in OVX rats produced responding similar to that of the intact females. Furthermore, there was no difference in the PTZ-discrimination dose response between two groups of OVX rats trained with PTZ before and after ovariectomy. This rules out the possibility that ovariectomy results in qualitatively different discriminative properties of PTZ. These findings suggest that estrogen is responsible, at least in part, for the difference in PTZ-lever responding in male and female rats.

Studies have suggested that female hormones modulate the GABAergic system and the anxiogenic stimulus. Direct involvement of estrogen and progesterone on GABAergic activity is shown in a study where both estrogen and progesterone resulted in an increase of [H]muscimol (a GABA_A agonist)-binding sites in selected brain areas of OVX rats (Maggi and Perez, 1984). Indirect evidence comes from a study in which ovariectomy abolished the sex difference in the threshold for PTZ-induced seizures by decreasing the threshold of female rats to a level similar to that of male rats (Kokka et al., 1992). In an anxiety model, adult female rats that received neonatal treatment with the estrogen antagonist tamoxifen or prepubertal ovariectomy showed a greater anxiogenic response than control females (Zimmerberg and Farley, 1993).

Progesterone, its metabolites (neuroactive steroids), and synthetic neurosteroids have been reported to have anxiolytic effects mediated by GABA_A receptors (Majewskia et al., 1986; Gee et al., 1987; Wieland et al., 1991; Beekman et al., 1998). Furthermore, progesterone withdrawal resulted in an anxiety-like stimulus in an anxiety model (Galloy and Smith, 1993). Neuroactive steroids also modulated the PTZ stimulus in a study where 3α,5α-tetrahydropregestosterone before significantly increased the PTZ seizure threshold dose (Finn and Gee, 1994). Sex differences have also been observed in the effect of neurosteroids on seizures. During EW, 3α,21-dihydroxy-5α-pregnan-20-one elevated seizure thresholds in female but not in male rats (Devaud et al., 1998).

Within our data, ovariectomy did not completely abolish the different response to PTZ in male and female rats, which suggests that other factors are also involved. In addition, sham female rats showed a cyclic variation in estrogen levels, but their performance in the PTZ discrimination was not cyclic. Other studies have reported no direct effect of ovarian factors or the estrus cycle on other anxiety-related effects. In the elevated plus maze, neither OVX nor estrus cycle modified anxiogenic behavior in rats (Nomikos and Spyraki, 1988). OVX did not influence ethanol-induced motor incoordination or spontaneous open field activity in mice (Becker et al., 1985). Forced swimming induced immobility in male rats to a greater extent than in female rats (Alonso et al., 1991). The immobility level was similar in the different stages of the estrous cycle of female rats. These data are consistent with the general hypothesis that periodic or continuous estrogen exposure at a threshold concentration of estrogen is necessary to maintain greater GABA_A neurotransmission in females than in males, but that acute increases in estrogen activity do not directly facilitate GABA_A function. In this context, an estradiol concentration at an estradiol surge in sham female rats may be critical to provide a hormonal milieu at its threshold concentration, which is significantly higher than that in OVX rats (data not shown).

The role of male hormones in anxiety-related behavior is less defined than that of female hormones. In general, male hormones appear to influence anxiety-associated behaviors during the developmental period rather than during adulthood (Zimmerberg and Farley, 1993; Astiningsih and Rogers, 1996; Lucion et al., 1996). The results of our experiments are consistent with those of other researchers that have determined that castration of adult male rats produces no significant change in anxiogenic response (Zimmerberg and Farley, 1993; Astiningsih and Rogers, 1996).

The observed sex difference in our study does not appear to be due to different blood levels of ethanol or PTZ. Given the same dose of ethanol, female rats have a higher (Sutker et al., 1983) or a similar BEC (present study, data not shown) than male rats, and the ethanol clearance rate does not differ between male and female rats (data not shown). Similarly, the half-life of PTZ does not differ between male (Esplin and Woodbury, 1956) and female rats (Ramzan and Levy, 1985). In addition, there was no male-female difference during the acquisition phase of the PTZ discrimination task. These data argue against the possibility that the lower intensity of PTZ and EW-induced PTZ-like stimuli in sham female rats is due to a lower PTZ level and BEC than male rats.

In summary, these findings demonstrate that: 1) female rats are less sensitive to the anxiogenic discriminative stimulus effects of PTZ; 2) female rats are less sensitive to the anxiogenic effects of EW as measured by PTZ-lever responding; and 3) estrogen plays some role in mediation of these sex differences. These findings suggest that the development of separate clinical strategies for the treatment of anxiety disorders related with estrogen abuse in men and women may be important.

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References