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Sex Differences in Sensitivity to Pentylenetetrazol but Not in $GABA_A$ Receptor Binding

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KOKKA, N., D. W. SAPP, U. WITTE AND R. W. OLSEN. *Sex differences in sensitivity to pentylenetetrazol but not in $GABA_A$ receptor binding.* PHARMACOL BIOCHEM BEHAV 43(2) 441–447, 1992.—Female rats have a higher threshold than males for seizures induced by the convulsant pentylenetetrazol, a $GABA_A$ receptor–chloride channel complex blocker. No sex difference was observed for the anticonvulsant activities of ethanol or diazepam to protect against pentylenetetrazol seizures. Ovariectomy reduces the pentylenetetrazol seizure threshold of females to that of males. In contrast, females have a lower threshold than males to electroshock seizures. Pentylenetetrazol receptors were compared in males and females and gonadectomized animals by binding of several radioligands to the $GABA_A$ receptor complex. No differences were found for these four groups of animals in the binding of [^{3}H]flunitrazepam to the benzodiazepine sites and [^{35}S]*t*-butyl bicyclophosphorothionate ([^{35}S]TBPS) to the chloride channel/convulsant sites in membrane homogenates, nor for allosteric modulation of binding by GABA, the steroid anesthetic alphaxalone, or the benzodiazepine Ro 5-4864. In tissue section autoradiography, no difference was observed for these same assays nor for the binding of [^{3}H]muscimol in the presence and absence of alphaxalone in several major regions. We conclude that circulating female sex hormones, possibly neurosteroid metabolites of progesterone, known to interact directly with the $GABA_A$ receptor complex, are involved in the sex differences in pentylenetetrazol seizure susceptibility.

Sexual dimorphism Convulsants Anticonvulsants Autoradiography

SEX differences in sensitivity to the pharmacological effects of drugs acting on the $GABA_A$ receptor-chloride channel complex (GRCC) have been reported in several studies. The GRCC is a membrane protein in which open and closed chloride channel conformations are regulated by GABA, the most abundant inhibitory neurotransmitter in the brain. Receptors for benzodiazepines, barbiturates, and picrotoxin-like convulsants, also located on the GRCC, interact allosterically to regulate $GABA_A$ receptor chloride conductance [reviewed in (17)]. In addition, recent evidence of membrane receptors on the GRCC for a number of neuroactive steroids suggest that GABAergic activity is also directly modulated by steroid hormones and/or their metabolites (5,8,15,21,24).

Sex differences in GRCC-mediated pharmacology include reports that female rats show a greater hypothermic response than males to both benzodiazepines and barbiturates (26) and a greater sensitivity to benzodiazepine inhibition of plasma corticosterone levels (19). The anesthetic steroid alphaxalone is less potent in males than females, but estrogen treatment of the males for 10 days increases their alphaxalone sensitivity to that of the females (7). Sex differences in $GABA_A$ receptor binding following swim stress (1) and variation of the frequency of certain types of epileptic seizures with the menstrual cycle in female humans (2) suggest interactions between nervous system activity at the level of the GRCC and the adrenal and ovarian steroids. Male rats are more sensitive than females to behavioral abnormalities induced by prenatal exposure to chronic benzodiazepines (3), but female rats are more sensitive than males to the convulsant picrotoxin, a GRCC blocker (19). In a preliminary report (12), we observed that males and females differ in sensitivity to the GABA channel blocker pentylenetetrazol (PTZ): Males were more sensitive, with a significantly lower PTZ seizure threshold dose than females. Gonadectomy did not affect males, but lowered the female PTZ seizure threshold to that of the male. We thus investigated the possibility that the receptors for PTZ, the $GABA_A$ receptors (GRCC), or steroid modulation of the GRCC might be different between sexes or altered by gonadectomy. We also measured seizure threshold with electroshock (ES) for comparison with PTZ sensitivity because an earlier

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report indicated that female rats were more susceptible than male rats to convulsions induced with ES (31).

Recent molecular biologic developments have shown that the GRCC is an oligomeric structure consisting of five polypeptide subunits produced from cDNA clones for five or more subunit candidate and multiple subtypes of each (18). Different subunit combinations produce multiple GRCC subtypes that exhibit different ligand binding affinities and pharmacological specificity, including differential sensitivity to steroid modulation (13,14,16–18,20,23). Thus, sex differences in GABAergic sensitivity, resulting from differences in receptor subtypes or in their distribution and density in the brain, would seem feasible. Shephard et al. (22) reported preliminary findings of small sex differences in benzodiazepine receptor binding in some brain regions of Roman rats. In this study, binding of the channel ligand [^{35}S]TBPS, the GABA ligand [^{3}H]muscimol, and the benzodiazepine ligand [^{3}H]flunitrazepam, plus allosteric modulation by GABA and the neuroactive steroid alphaxalone and the novel benzodiazepine Ro 5-4864, were examined.

METHOD

PTZ Seizure Threshold Determination

All experiments were performed on female and male Sprague-Dawley rats initially weighing 180–220 g. Rats were housed in a vivarium under a 12 L : 12 D cycle and had free access to food and water. PTZ seizure threshold was determined by IV injection of PTZ, 10 mg/ml in saline. PTZ was injected slowly into the tail vein of rats held under light restraint until the onset of seizures. The endpoint of injection was one to three jerks at the base of the tail, which reliably preceded and signaled the onset of clonic or tonic seizures. Rats were placed in clear plastic cages, 11 × 18 × 10 in., and observed for clonic or tonic seizure activity. Repetitive and jerking movements of forelimb and head and body spasms were recorded as clonus; ventroflexion, extension of forelimbs, and flexion of forelimbs with extension of hindlimbs were recorded as tonus. Fatality for this method of determining seizure threshold by IV administration of PTZ was approximately 5%.

ES Seizure Threshold Determination

ES seizure thresholds were determined in 40 female and 40 male rats by the up-and-down method, which is designed for sensitivity testing in all or none assays (6). In this method, current intensity is raised a fixed amount if a shock did not produce a seizure in the preceding rat or lowered the same amount if a seizure was produced; five to seven rats are required for each ES seizure threshold determination. From the pattern of seizure-no seizure responses, ES seizure threshold is calculated from the following: ES threshold = $X + K_d$, where X = last shock (log mAmp), K = tabular value which is determined by the pattern of responses, and d = shock interval (log mA). Tests in rats have shown this method to be reliable and simple to perform with ear clip electrodes for reproducible threshold measurements of seizure sensitivity.

Effects of Gonadectomy

The effect of gonadal status on seizure sensitivity was determined by measuring PTZ seizure threshold before and after gonadectomy. Seizure threshold was first determined in intact female and male rats that were next gonadectomized 3 days later. The ovaries were removed through two small abdominal incisions after ligation of attached blood vessels and the testes were removed through a scrotal incision. Following a 10-day recovery period, PTZ seizure threshold was measured in both groups to determine the effects of gonadectomy.

Anticonvulsant Effects of Ethanol and Diazepam

The anticonvulsant effects of ethanol and diazepam were measured in female and male rats to determine whether a sex difference in seizure inhibition could be demonstrated. For these studies, ethanol was given 60 min and diazepam 30 min before testing with PTZ; control rats were given saline and vehicle, respectively. Statistical analysis of mean differences between treatment groups was performed with Student's t-test; $p < 0.05$ was considered statistically significant.

Radioligand Binding Assays

Membrane homogenates—[^{35}S]TBPS binding. The membrane preparation followed King et al. (11). Frozen rat brain hemispheres (four halves per group) were homogenized in 0.32 M sucrose, then centrifuged for 10 min at 1,000 × g. The supernatant was centrifuged at 100,000 × g for 45 min. This pellet was osmotically disrupted in distilled water, then washed twice with Tris-HCl buffer (50 mM, pH 7.4), then dialyzed overnight against 1 mM EDTA (25) and washed twice in assay buffer (20 mM KH_2PO_4, 200 mM KCl, pH 7.5). For assay, membrane homogenate at 1.0 mg/ml protein was incubated for 90 min at 22°C with [^{35}S]TBPS [5 nM, 87–112 Ci/mM, New England Nuclear (NEN), DuPont, Boston, MA]. Nondisplaceable background was determined in the presence of 0.2 mM picrotoxinin (Sigma Chemical Co., St. Louis, MO) and these values were subtracted from the total binding to calculate specific binding. Concentration-dependent allosteric modulation by GABA, alphaxalone, and Ro 5-4864 were measured 8–10 concentrations of these drugs in triplicate. Samples were collected by vacuum filtration (Whatman GF/B) and the filters counted for radioactivity in Cytoscint (ICN, Irvine, CA).

[^{3}H]Flunitrazepam binding. Membranes were prepared as above but without dialysis, and stored at −70°C. For assay, the membranes were thawed and washed twice in assay buffer (10 mM KH_2PO_4/K_2HPO_4, 100 mM KCl, pH 7.5). The incubation time was 60 min at 0°C, with protein concentration of 0.8 mg/ml. [^{3}H]Flunitrazepam was 1 nM, 103.2 Ci/mM (NEN-DuPont). Concentrations of GABA or alphaxalone were included in triplicate. Assays were terminated by filtration as above.

Semiquantitative autoradiography. Slide-mounted unfixed rat brain sections were thawed and preincubated in assay buffer for 30 min at 4°C, then incubated for 90 min at 22°C (TBPS, 30 nM), 60 min at 4°C (flunitrazepam, 0.5 nM), or 30 min at 4°C ([^{3}H]muscimol, 10 nM) without and with various concentrations of inhibiting or enhancing drugs as previously described (16). Nonspecific background was determined with parallel assays including 50 μM picrotoxinin, 1 μM clonazepam, or 10 μM GABA, respectively, and was negligible (not shown). The assay buffers were 0.12 M NaCl, 50 mM Tris-HCl, pH 7.5, (TBPS), plus 1 mM EDTA for preincubation but not binding incubation, 0.17 M Tris-HCl, pH 7.4 (flunitrazepam), or 0.1 M KCl, 0.01 M K-phosphate, pH 7.4 (muscimol). Postassay rinses in the same buffers were 2 × 5 min for TBPS, 2 × 30 s for flunitrazepam, and 2 × 5 s for muscimol. In other parallel assays were included various concentrations of several nonradioactive substances that inhibit or en-

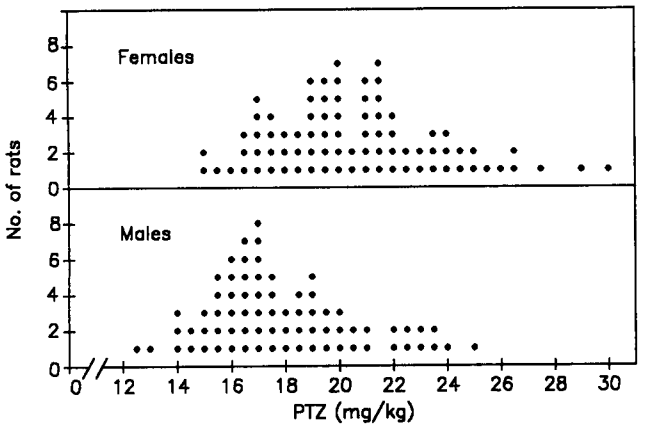

FIG. 1. Sex difference in PTZ seizure threshold. Mean PTZ seizure threshold of female rats was 20.7 ± 0.4 mg/kg (n = 80) and of male rats 17.7 ± 0.3 mg/kr (n = 59); statistical significance between groups was $p < 0.01$.

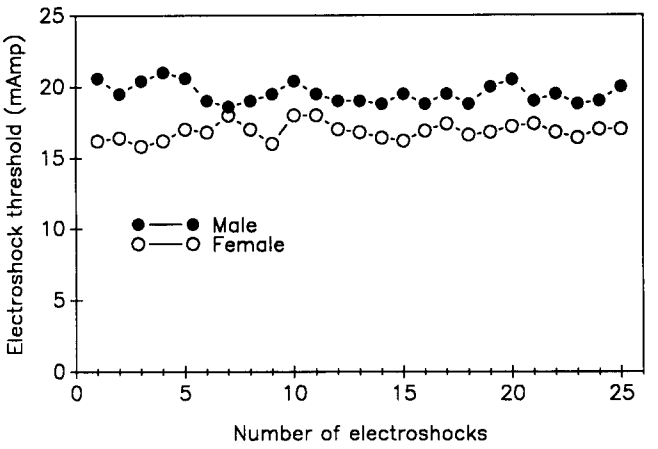

FIG. 3. Effects of daily electroshocks on ES seizure threshold. Seizure threshold in eight female (○) and eight male rats (●) was determined daily for 25 days.

hance binding. After binding, rinsing, and drying the tissue sections, autoradiograms were generated by exposure of [^{3}H]-sensitive ultrafilm (Pharmacia-LKB, Pleasantville, CA) to the radioactive tissue sections for 60 days (TBPS, muscimol) or 14 days (flunitrazepam), including [^{3}H]-embedded plastic microscales (Amersham Corp., Arlington Heights, IL). Computer-assisted microdensitometry was performed with a diode array camera/image analyzer (Technology Resources, Nashville, TN). Each point (total or background) was the mean of at least three sections (both hemispheres on frontal sections) for each anatomical subregion, and the experiments were repeated on at least three animals. When IC_{50}/EC_{50} values were calculated (graphically), at least two experiments yielding measurements for at least five of the seven concentrations of modulatory drugs tested were employed. The basal binding has been measured by Scatchard plot for several brain regions on numerous occasions (not shown), with average K_d values of 30 nM (TBPS), 15 nM (muscimol), and 2.5 nM (flunitrazepam), identical for six brain regions (4,16).

RESULTS

Males and Females Differ in PTZ Seizure Threshold

A scattergram is shown in Fig. 1 of all PTZ seizure threshold determinations in rats that served as controls in experiments related to alcohol dependence, chemical kindling, and anticonvulsant drugs. Although considerable overlap was found, the results showed a small but significant sex difference in sensitivity to PTZ-induced seizures: PTZ seizure threshold was 20.7 ± 0.4 in females and 17.7 ± 0.3 in males ($p < 0.01$).

Sex Differences in ES Seizure Threshold

Typical patterns of seizure responses as determined by the up-and-down method for small samples are shown in Fig. 2. The criteria for determining clonic and tonic seizures with ES were the same as those used for PTZ. Measurements of seizure threshold in a large group of rats, in which each rat was tested

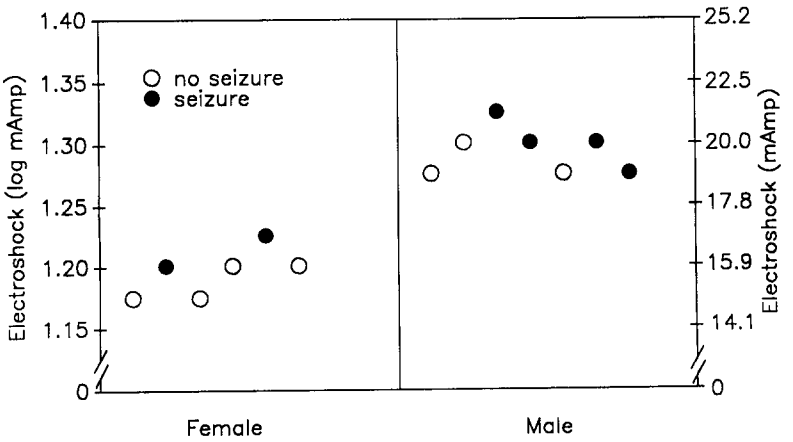

FIG. 2. Sex difference in ES seizure threshold determined by the up-and-down method. (●), seizures; (○), nonseizures.

TABLE 1

SEX DIFFERENCE IN PTZ SEIZURE THRESHOLD EFFECTS OF GONADECTOMY AND ETHANOL

	PTZ (mg/kg, IV)	
Pretreatment	Male	Female
None	(7) 16.6 ± 0.6	(7) 22.3 ± 0.9
Gonadectomy	(7) 15.6 ± 0.4	(7) 15.9 ± 0.9
Ethanol, -60 min		
0.75 g/kg IP	(6) 22.1 ± 0.8	(6) 25.8 ± 1.5
1.50 g/kg IP	(6) 31.7 ± 0.7	(6) 33.5 ± 1.6

PTZ seizure thresholds following gonadectomy or ethanol administration were determined as described in the Methods section. Values are means ± SEM; number of rats shown in parentheses.

once only, showed that female rats have a greater seizure susceptibility to ES seizures than male rats; ES seizure threshold was 16.4 ± 0.5 mAmp in females and 19.4 ± 0.4 mA in males ($p < 0.01$). Six threshold determinations were done for each sex.

When the effect of repeated testing on ES seizure threshold was measured, no change was seen in eight female and eight male rats that were stimulated daily for 25 electroshocks. The results in Fig. 3 show that ES seizure threshold for both females and males was stable because daily variations were less than 10% and ES thresholds after 25 shocks were close to the starting values.

Effects of Gonadectomy

To determine the contributions of the ovaries and testes to the difference in seizure sensitivity, PTZ seizure thresholds were measured in female and male rats before and after they were gonadectomized. The results in Table 1 show that prior to gonadectomy PTZ seizure threshold was 22.3 mg/kg PTZ for females and 16.6 mg/kg for males. At 10 days following gonadectomy, PTZ seizure threshold of females decreased to that of males, which showed no change (Table 1). These results indicate that the ovaries were mainly responsible for the sex difference in sensitivity to PTZ seizures.

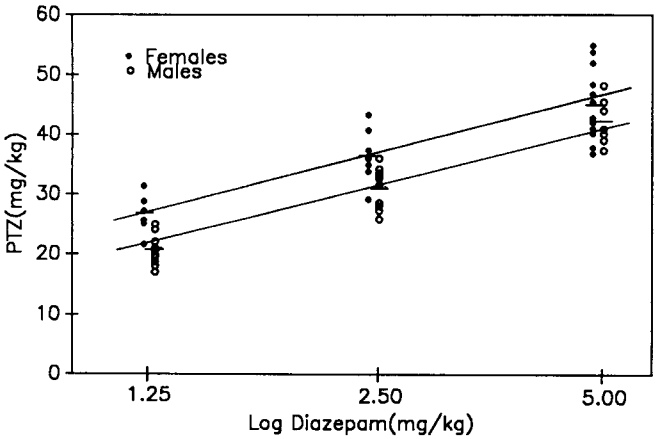

FIG. 4. Diazepam antagonism of PTZ seizures in female and male rats. Horizontal bars represent mean PTZ seizure threshold of each diazepam treatment group.

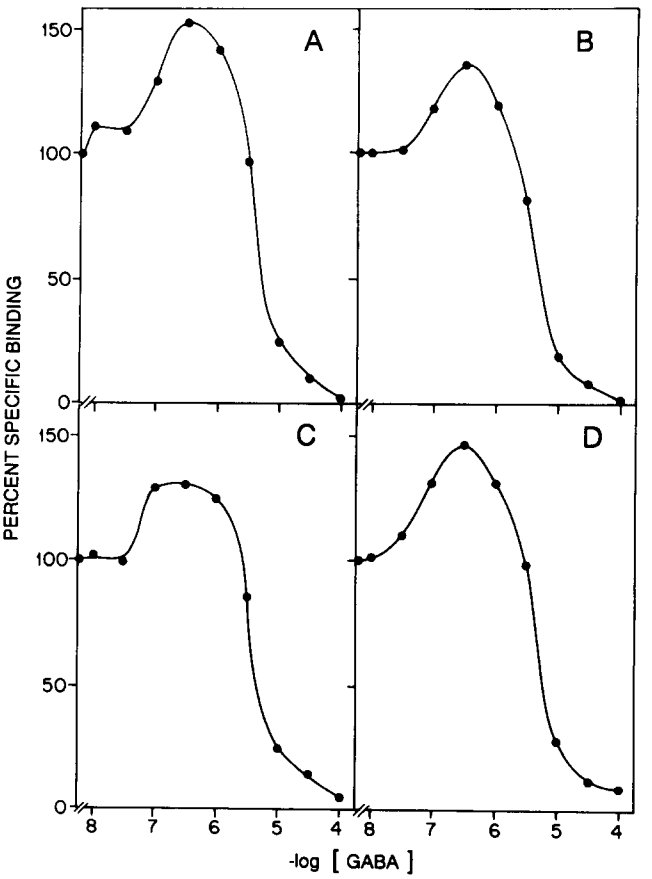

FIG. 5. Modulation by GABA of [^{35}S]TBPS binding in brain membrane homogenates of normal and gonadectomized male and female rats. TBPS binding was assayed as described in the Method section and the concentration dependence of GABA modulation determined. The results shown are the mean of triplicate assays and two separate experiments. A, males; B, gonadectomized males; C, females; D, gonadectomized females.

Males and Females Do Not Differ in Ethanol and Diazepam Antagonism of PTZ Seizures

The effects of alcohol and diazepam on PTZ seizure threshold also were measured in female and male rats to determine whether a sex-linked difference in sensitivity to their anticonvulsant action could be shown. Although ethanol had considerable anticonvulsant potency, no difference in sensitivity between females and males was seen (Table 1). Diazepam had potent anticonvulsant activity, as shown in Fig. 4, but the parallel dose-response curves show that the increases of PTZ seizure threshold were the same for both females and males. These data show that female and male rats did not differ in their sensitivity to ethanol and diazepam antagonism of PTZ seizures.

GABA-Benzodiazepine Receptor Binding Does Not Vary Between Sexes

Membrane homogenates. Total rat brain (one hemisphere from each of four animals per group pooled) particulate fractions were assayed for the binding of [^{35}S]TBPS, including modulation by GABA, the steroid anesthetic alphaxalone, and

the novel excitatory benzodiazepine Ro 5-4864 (10), and the binding of [^{3}H]flunitrazepam, including modulation by GABA and alphaxalone. No significant differences were found for these five parameters in the four sex groups: normal and gonadectomized males and females.

Figure 5 shows the concentration-dependent modulation by GABA of [^{35}S]TBPS binding to well-washed, dialyzed membranes for the four groups. A biphasic curve was found for all four, with enhancement of 30–50% at 0.1–1 μM and inhibition at higher concentrations, reaching complete inhibition at 100 μM. The shapes of the curves, maximal extent of enhancement, and concentrations at which enhancement and inhibition occurred were similar for the four groups, with variation of less than 20%. Figure 6 indicates concentration-dependent modulation of [^{35}S]TBPS binding by the steroid alphaxalone, assayed in the absence of GABA. A biphasic curve was also observed; however, the enhancement was more marked, reaching about 100% (200% of control) at 10 μM steroid, then declining slightly at 100 μM.

Figure 7 (upper) shows concentration-dependent modulation of [^{35}S]TBPS binding (assayed in the absence of GABA) by Ro 5-4864 in male (A) and female (B) rats. Gonadectomized animals gave identical results. The benzodiazepine inhibited the binding down to a plateau of about 50% of control, producing half the maximal effect at about 1–2 μM. Figure 7 (lower) shows concentration-dependent modulation by GABA of [^{3}H]flunitrazepam binding to female (A) and gonadectomized female rat brain (B). Male rats, normal, or gonadectomized, gave patterns identical to A. GABA enhances the binding up to a maximum of 45–55%. The gonadectomized females had a slightly more potent effect of GABA ($EC_{50} \simeq 0.2$ μM) than other animals ($EC_{50} \simeq 1$ μM) but this was not statistically significant by t-test. Alphaxalone (0.1–100 μM) had no effect on [^{3}H]flunitrazepam binding to membranes of any of the four groups of rats.

Tissue section autoradiography. The same five assays, plus [^{3}H]muscimol binding without and with alphaxalone, were performed on frozen, unfixed tissue sections of rat brain to examine individual anatomic regions for the different sex groups. Table 2 shows selected measurements for binding of these ligands and allosteric modulation in several brain regions for the four groups of animals. The binding of TBPS, flunitrazepam, and muscimol were compared in the four groups

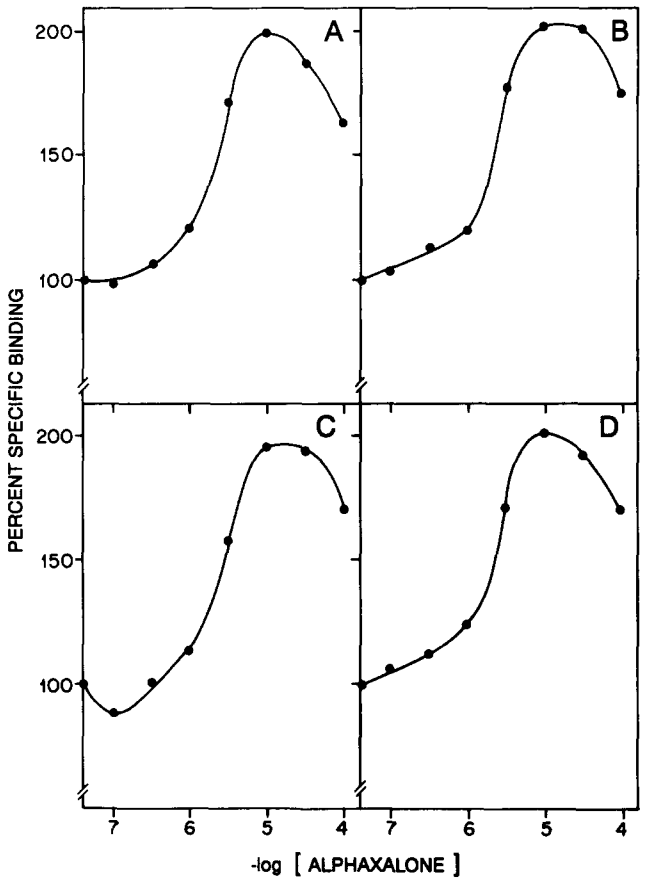

FIG. 6. Modulation by alphaxalone of [^{35}S]TBPS binding in brain membranes homogenates from normal and gonadectomized male and female rats. TBPS binding was assayed as described in the Method section and the concentration dependence of alphaxalone modulation determined. The results are the mean of triplicate assays and two separate experiments. A, males; B, gonadectomized males; C, females; D, gonadectomized females.

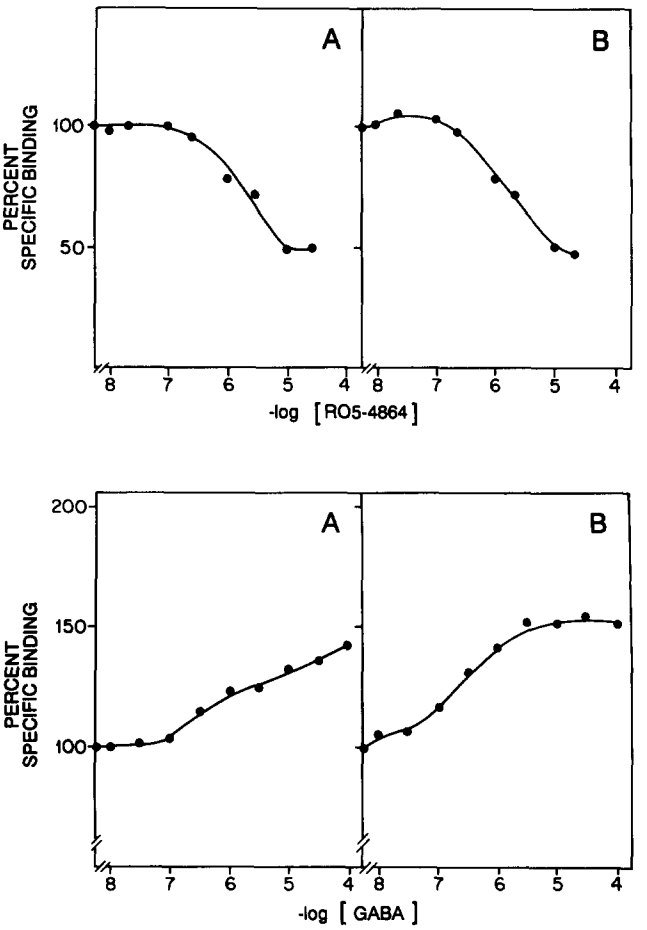

FIG. 7. (Upper). Modulation by Ro 5-4864 of [^{35}S]TBPS binding in brain membrane homogenates from male (A) and female (B) rats. (Lower). Modulation by GABA of [^{3}H]flunitrazepam binding to membrane homogenates from female (A) and gonadectomized female rat brains (B). The results are the mean of triplicate samples and two separate experiments.

TABLE 2

BRAIN REGIONAL VARIATION OF [^{35}S]TBPS, [^{3}H]FLUNITRAZEPAM, AND [^{3}H]MUSCIMOL BINDING FOR FOUR SEX GROUPS AND MODULATION BY ALPHAXALONE

	Cortex		Hippocampus Dentate Gyrus		Inferior Colliculus		Cerebellum Granule Cell		Cerebellum Molecular Layer	
	Alone	+Fax	Alone	+Fax	Alone	+Fax	Alone	+Fax	Alone	+Fax
[^{35}S]TBPS										
Male, control	0.068	0.051	0.058	0.042	0.069	0.057	0.073	0.026	0.060	0.039
Male, GDX	0.065	0.056	0.061	0.049	0.070	0.060	0.075	0.029	0.058	0.044
Female, control	0.072	0.053	0.064	0.043	0.073	0.054	0.060	0.025	0.065	0.039
Female, GDX	0.070	0.047	0.063	0.042	0.069	0.048	0.067	0.026	0.062	0.037
[^{3}H]Flunitrazepam										
Male, control	8.05	10.77	6.79	9.59	7.62	10.54	1.33	1.40	4.91	7.15
Male, GDX	8.86	11.11	8.02	9.43	7.80	8.76	1.32	1.42	5.34	6.62
Female, control	9.05	12.32	8.94	10.92	7.72	9.81	1.26	1.61	5.57	6.85
Female, GDX	9.00	12.34	8.30	9.78	7.23	9.14	1.21	1.89	5.09	6.84
[^{3}H]Muscimol										
Male, control	10.06	11.75	7.28	7.89	9.38	11.57	23.25	22.90	7.93	9.55
Male, GDX	10.05	11.39	8.10	7.99	9.24	10.94	25.41	24.34	6.87	7.94
Female, control	8.89*	9.55*	6.72	7.19	8.26	10.57	19.98	19.24	6.43	8.04
Female, GDX	9.21*	9.64*	7.40	6.75	8.79	9.65	23.02	21.41	5.96	6.93

Binding of three different ligands in several brain regions from the four sex groups were measured using quantitative autoradiography. For each region listed above, specific binding is given on the left side and modulation by 10μM alphaxalone (+Fax) is listed on the right side of the column. Each value represents an average of measurements from four different tissue sections (four animals). Values for [^{35}S]TBPS are given in units of optical density and [^{3}H]flunitrazepam and [^{3}H]muscimol are in units of nCi/mg protein. The four groups were compared pair-wise by Student's t-test (*$p < 0.05$).

for cerebral cortex, hippocampus, inferior colliculus, and cerebellar cortex, and also alphaxalone modulation of the three ligands in selected areas. No significant differences (t-test, $p < 0.01$) in receptor binding were found for any of these assays in any of the major brain regions for the four sex groups. A small difference ($p < 0.05$) between males and females was found in muscimol binding in cortex.

DISCUSSION

The present results show a sex difference in susceptibility of rats to both PTZ- and ES-induced seizures. The female-male difference determined with PTZ, however, is opposite to that previously reported by Woolley et al. (31) for ES and confirmed in the present study. These results indicate that different neural substrates mediate the convulsive responses to PTZ and ES. Woolley and Timiras (29,30) also reported that ES seizure threshold varied with the estrous cycle and concluded from their studies with hormone treatment that estrogens were primarily responsible for the enhanced sensitivity of female rats.

The sex difference in susceptibility of rats to PTZ-induced seizures could result from sexually dimorphic differences in the binding of PTZ to the GRCC. Our results, however, show that the GRCC are not different in males and females, at least in the ligand binding activities, allosteric properties, and brain regions examined. While sexually dimorphic organizational differences in the nervous system may be responsible, the effect of ovariectomy to reduce female PTZ seizure threshold to that of males suggests more rapid activational or direct actions of female steroid hormones on the function of the GRCC. The lower sensitivity of females might be attributed to the increased amounts of steroid hormones released during the estrous cycle; some of their metabolites, especially progesterone metabolites, have been shown to exhibit stereoselective binding to the GRCC to enhance GABA activity through a direct membrane, nongenomic action (5,9,15,24,28). Because of the importance of steroid hormones in homeostatic regulation, the presence of steroid receptors on the GRCC may have significant implications related to the control of brain excitability. Due to the presence of steroid sex hormones and metabolites, females might show a cyclical variation in "GABA tone," consistent with periods of CNS depression, high sensitivity to sedative-hypnotic-anesthetic drugs, and low sensitivity to anxiogenesis and low seizure susceptibility. Ovariectomy would eliminate both the cyclical variations in levels of neuroactive steroids and, thereby, the difference in PTZ seizure sensitivity, as observed in the present study. Woolley and Timiras (30), in contrast, have shown that ES seizure threshold of mature rats was unaffected by ovariectomy, suggesting again that the processes underlying the sex difference in seizure susceptibility to PTZ and ES are not the same.

Although our results would be in accord with the involvement of neuroactive progesterone metabolites, Fink et al. (7) suggest an alternative explanation for sex differences in GABA-mediated activity. A sex difference was shown in rats in sensitivity to alphaxalone, a steroid anesthetic that binds to the same sites on the GRCC as the natural steroids (9,15,28). Male rats were found to be less sensitive than females to alphaxalone; following gonadectomy and implant of estrogens, however, the dose of alphaxalone required for anesthesia in males was reduced to that of females. The enhanced sensitivity of estrogen-treated male rats to its anesthetic effects could be attributed to an increase of steroid-modulated GRCC activity brought about by the estrogen implant. Further, because several days were required for the estrogen implants to increase sensitivity to alphaxalone of male castrates to that of females it is likely that the estrogen effects were mediated by a genomic

mechanism. Thus, the lower sensitivity to PTZ and the higher sensitivity to alphaxalone of female rats could be due to enhanced GABA activity at membrane GABA receptors that have modulatory steroid receptor sites directly on the GRCC. GABA activity could be augmented selectively in the short term by neurosteroids acting at those receptors that, in turn, are under longer-term regulatory influence of estrogens or other hormones.

In contrast to the present findings, however, is the report of Pericic et al. (19) that the incidence of convulsions and deaths following picrotoxin was higher in female than in male rats. Because the convulsant symptoms and mechanism of action of PTZ are similar to that of picrotoxin, our results showing a higher seizure threshold for PTZ in females would seem to be in direct contrast to those showing a higher incidence of seizures and death with picrotoxin in females. Gender differences in seizure susceptibility to picrotoxin were also studied by Thomas (27), who reported that seizure susceptibility of female and male rats was dependent upon both seizure type and stimulation of intensity. Our results with *threshold* doses of PTZ and those of Pericic et al. (19) with *maximal and lethal* doses of picrotoxin would seem to be in accord with Thomas' findings (27) that sex differences in susceptibility to seizures are most evident with doses of minimal and maximal stimulation and that an increased sensitivity to one seizure type does not establish an increased susceptibility to all seizure types.

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