Pharmacology Biochemistry and Behavior, Vol. 66, No. 4, pp. 751–764, 2000

0091-3057/00/$–see front matter

PII S0091-3057(00)00239-2

The Pharmacodynamics of PK 11195 in Diazepam-Dependent Male and Female Rats

J. W. SLOAN, E. P. WALA, X. JING AND J. R. HOLTMAN, JR.

Department of Anesthesiology, University of Kentucky, Lexington, Kentucky, 40536

Received 04 January 2000; Revised 24 January 2000; Accepted 01 February 2000

SLOAN, J W., E. P. WALA, X. JING AND J R. HOLTMAN, JR. *The pharmacodynamics of PK 11195 in diazepam-dependent male and female rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 751–764, 2000.—These studies were undertaken to 1) determine whether repeated dosing with the peripheral benzodiazepine antagonist PK 11195 alters its ability to precipitate withdrawal abstinence in diazepam-dependent rats; 2) whether the administration of PK 11195 and the central benzodiazepine antagonist, flumazenil, 3 days apart to the same rat produces an ordering effect in the intensity of withdrawal abstinence; 3) whether there are gender differences in these effects. Age-matched male and female Sprague Dawley rats had capsules implanted weekly that contained approximately equal (mg/kg) doses of diazepam (120 and 90 mg, respectively) or empty capsules (controls). After 5 implants, the maximum precipitated withdrawal score (PAS_{MAX}) induced by PK 11195 and/or flumazenil (10 mg/kg/IV, respectively) was measured. Repeated administration of PK 11195 (1x/day for 5 days) induced tolerance with regard to the intensity of the PAS_{MAX} and with gender-related differences. When PK 11195 was administered weekly (5 weeks), rather than daily, tolerance did not develop in either sex. The PK 11195- and flumazenil-induced PAS_{MAX} was not changed by the order in which they were administered. There were gender differences in that females had a higher PAS_{MAX} after flumazenil than after PK 11195 and vocalized more after all treatments than males. © 2000 Elsevier Science Inc.

Gender Tolerance to PK 11195 Diazepam-dependent rats Peripheral benzodiazepine receptors Pain Flumazenil Precipitated withdrawal

APPROXIMATELY 12.5% of the world population (more women than men) is prescribed a drug to reduce anxiety during the course of a year, most of which are benzodiazepines (BZs) (92). The BZs exert their effects through central (CBR) and peripheral BZ receptors (PBR) heterogeneously distributed throughout the body. The CBR were originally designated as 2 distinct subtypes: Type I (now called CBR1 [BZ1 or φ_1]) and Type II (now called CBR2 [BZ2 or φ_2]). Although the CBRl/CBR2 class;ification is somewhat of an oversimplification, it does have a sound structural basis. The CBR, an integral part of the $GABA_A$/chroride ionophore complex, are formed when α- and β-subunits are co-expressed with the γ2-subunit. It is fairly well established that the heterogeneity of the CBR binding sites arises mainly from the existence of different $GABA_A$ α-subunits (α1, α2, α3, or α5)(60,61). Although the CBR make up the major population of binding sites in the brain, the PBR localization belies the name and are also heterogeneously distributed in the brain, as well as in the periphery, generally on the outside membrane of the mitochondria. The brain PBR(s) have typically been associated with glial cells (mainly) and ependymal cells, although, more recently, they have been found to have a neural localization as well. The PBR is a small monomeric protein composed of the ~18-kD binding site for the specific antagonist, PK 11195 [1-(2-chlorophenyl)-N-methyl-(1-methylpropyl)-3-isoquinolinecarboxamide] and for the specific agonist, Ro 5-4864 (4'chlordiazepam) (13,35). A 36-kD peptide with structural and functional similarities to a voltage-dependent anion channel (VDAC) has been cloned and sequenced but has no homology to the 18-kD peptide suggesting the PBR is not a subunit of a larger VDAC. This does not rule out the possibility, however, that the PBR and the VDAC are somehow structurally or functionally linked (13). The PBR can alter the rate of steroidogenesis by altering the rate of cholesterol transport to the mitochondrial cytochrome $P\text{-}450_{SCC}$ enzyme and may modulate the production of endo-

Requests for reprints should be addressed to Jewell W. Sloan, PhD, Department of Anesthesiology, University of Kentucky College of Medicine, 800 Rose Street, Lexington, Kentucky 40536-0293; Tel.: (606) 323-8034; Fax (606) 323-1924; E-mail: sjewell@pop.uky.edu
This work was supported by the University of Kentucky Research Fund.

geneous brain steroids by glial cells as well (24,40,62,66,86). The P-450$_{SCC}$ enzyme is responsible for catalyzing the conversion of cholesterol to pregnenolone, the rate-controlling step in sterodogenesis. Further, the finding that several steroid metabolites such as 3α-hydroxy-5α-pregnan-20-one and 3α,21-dihydroxy-5α-pregnane-20-one alter the GABA$_A$ receptor complex suggests a possible link between the CBR/GABA$_A$ complex and the PBR (13,53,62) although the details for such an interaction are not elucidated. Others have suggested that the intrinsic actions of PK 11195 in doses higher than necessary to saturate the receptors may act on a low-affinity site on the GABA-BZ receptor complex (17). The PBR may be distinguished from the CBR by the specificity of the binding properties of several BZ ligands to these receptors. For example, diazepam (DZ) binds with relatively high affinity to both central (CBR1, CBR2) and peripheral (PBR) receptors. The BZ antagonist, flumazenil, binds to CBR1 and CBR2 but not to PBR, whereas PK 11195, the specific PBR antagonist, binds with high affinity to the PBR but not to the CBR (40,42,56,76 for review).

Flumazenil reverses all the CBR- but not the PBR-mediated effects of an acute dose of a BZ agonist (45), but does not precipitate withdrawal after an acute dose of DZ (44,90). After prolonged exposure to BZs, flumazenil antagonizes the central effects of BZs and can unmask all signs of withdrawal that are mediated through the GABA$_A$/CBR/ionophore complex (93).

Little is known about the interaction of PK 11195 with an acute dose of DZ. PK 11195 has a high affinity for the PBR, and, in low doses, acts as an antagonist of the convulsant and proconflict actions of the PBR agonist, Ro 5-4864, whereas in high doses it may have agonistic actions (12,16,52). The binding of [^{3}H] PK 11195 to rat cerebral cortex is displaced by DZ (1) with an order of potency of PK 11195>Ro 5-4864>DZ>clonazepam (3). Recently, it has been shown that PK 11195 induces a withdrawal syndrome in DZ-dependent subjects after either systemic or focal injections into different brain structures that reveals some of the same signs induced by flumazenil, but with marked qualitative and quantitative differences (50,69,70,78,81). Further evidence of the involvement of the PBR in the dependence-producing properties of the BZs is demonstrated by the attenuation of the withdrawal syndrome in mice by chronic co-administration with lorazepam (51).

Although many functions have been attributed to the PBR, it has only recently been found to have a physiologic and pharmacologic role. In addition to the role in the regulation of steroidogenesis, the PBR also play a role in mitochondrial respiration. This produces many cellular effects including alterations in cell growth and differentiation and calcium regulation. The PBR are also involved in the actions of BZs in modulating the body's immune reaction (39,40,57,66).

The specific PBR antagonist, PK 11195, is the ligand of choice in studies of the biochemical and physiologic properties of the PBR. It is used in studies of steroidogenesis in which it has been found to affect the release of cortotropin-releasing factor and ACTH in the rat as well as to directly elevate plasma corticosterone levels and to stimulate testosterone secretion from crude testicular Leydig cell preparations (40). PK 11195 significantly reduces Ro5-4864-induced convulsions in mice (to which tolerance does not develop); has no anticonvulsant action against picrotoxin; acts as a proconvulsant (to which tolerance develops) when combined with subconvulsant doses of picrotoxin and strychnine, but not when combined with pentylenetetrazole (16). PK 11195 increases the seizure threshold of Ro 5-4864 in El mice, an animal model of epilepsy (55). Further, by the use of [^{3}H]-PK 11195, it has been shown that the PBR numbers are altered in a variety of disease states including neuropsychiatric disorders such as schizophrenia in which platelet PBR are decreased by neuroleptic treatment; increased after acute and decreased after chronic exposure to stress in humans; altered by the chronic use of alcohol; cocaine; barbiturates, and BZs; increased in post-mortem tissues from patients with several neurological diseases such as Alzheimers; Huntingtons; multiple sclerosis; and in the periphery of infarcted areas of the brains of stroke victims; increased in brain tumors; in ovarian carcinomas; in colonic and prostatic adenocarcinomas; after tissue injury within the central nervous system (CNS) (cf 2,23,31,54). The PBR concentration in these many disease states holds much clinical interest.

Now that it is known that the PBR regulate steroidogenesis, greater consideration must be given to this function as it relates to the pharmacologic actions of drugs, particularly anxiolytics. The role of the PBR in steroid biosynthesis could contribute to differences in pharmacologic profiles and tolerance of various BZs and provide a rationale for alternative consideration in their therapeutic use as well as help to direct the design of anxyiolytics with greater specificity (cf 40). Because PK 11195 is the most frequently used drug for assessment of the effects of BZs at the PBR it is important to have an understanding of its pharmacodynamic, pharmacokinetic, and gender effects.

The therapeutic effects of the BZs are generally thought to be mediated by the CBR; however, recent studies have clearly shown that the PBR are also involved in many actions of the BZs and may play a role in the emergence of many unwanted side effects, including tolerance and dependence. The role of the PBR in BZ dependence is an area that is poorly understood and one that has not been extensively explored. Gender-related differences in precipitated withdrawal in DZ-dependent rats have been reported after IV infusion of flumazenil in male (90) and female (49) rats; after IP flumazenil in DZ-dependent male and female mice (59). Most chronic studies of DZ have been conducted in males or in orchidectomized male and ovariectomized female rats, whereas little attention has been directed toward DZ's dependence-producing effects in intact females. In view of the large body of evidence that the PBR are the critical factor in the rate-limiting step of steroidogenesis; that the PBR as well as the CBR are tonically and physically regulated by neural and hormonal mechanisms; that stress affects both the CBR and the PBR; DZ can alleviate some of these stress-induced changes which, in turn, are modified by gonad-related factors; and more women than men use BZs, emphasizes the importance of studying DZ dependence in both sexes (5,13,15,24,88,89).

It has been clearly demonstrated that tolerance does not develop to flumazenil's antagonistic effects when it is repeatedly administered in weekly intervals or longer to DZ-dependent subjects (25,46,93), whereas some tolerance appears to develop when it is administered at shorter intervals of 3 days or less to DZ-dependent baboons and rhesus monkeys (21,41). This important issue has not been extensively investigated with regard to PK 11195. Further, because PK 11195 and flumazenil have specificity for different BZ receptor subtypes, it was reasoned that these two antagonists could be administered to the same rat without interacting with each other when dosing is separated by intervals of 3 days. Therefore in view of a lack of information on these important aspects of the DZ precipitated withdrawal syndrome, the first objective of these experiments was to determine whether tolerance de-

velops to the ability of PK 11195 to precipitate a withdrawal syndrome in DZ-dependent rats and whether there are gender-related differences in this regard when PK 11195 is administered in either daily or in weekly intervals. The second objective was to determine if the order in which flumazenil and PK 11195 are administered alters the precipitated withdrawal effect in male and female DZ-dependent rats. The third objective was to determine the consequences of the combined administration of PK 11195 and flumazenil on the precipitated withdrawal syndrome and whether there are gender differences.

METHOD

Animals

Experiments, approved by the Institutional Animal Care and Use Committee of the University of Kentucky, were performed in age-matched (~90 days old) male (~350 g) and female (~250g) Sprague Dawley rats. The rats were housed individually in accordance with the NIH Guide for the Care and Use of Laboratory Animals in a climate-controlled facility with a 12 L/12 D cycle (lights on 0600 h) and had free access to standard laboratory chow and water.

Drugs and Chemicals

PK 11195 was purchased from Research Biochemical International. Flumazenil and DZ were generous gifts from Hoffman-LaRoche. PK 11195 and flumazenil were dissolved in dimethylsulfoxide (DMSO) which, along with HPLC standards, was purchased from Sigma, St. Louis, MO, USA. Solutions were freshly prepared on the day of experiments and protected from light.

Chronic Administration of DZ

Male and female rats acclimated to their surroundings and while under ketamine anesthesia (40 mg/kg) and sterile conditions had silastic capsules containing diazepam or empty capsules (control rats) implanted subcutaneously in the back. Male and female rats were administered approximately equal (mg/kg) doses of DZ (120 and 90 mg/kg, respectively) according to previously described procedures (22) with minor modifications (49). Initially, 2 silastic capsules were implanted in the back of each rat. Thereafter, a single capsule was implanted at weekly intervals. Capsules were not removed during the study to avoid complicated surgery. Body weight (BW) was recorded prior to the first capsule implantation, just prior to each subsequent implantation, then before, and one day after each IV injection of antagonist.

Experimental Groups

Three groups of male and female rats were treated chronically with DZ or empty capsules for 5 weeks prior to experimentation. New capsules were implanted throughout the experiments at weekly intervals. *Group I, daily injections of PK 11195 (10 mg/kg every 24 h) for 5 days:* this group consisted of 6 male and 6 female rats treated with DZ and 4 males and 4 female rats implanted with empty capsules. *Group II, weekly injections of PK 11195 (10mg/kg each week) for 5 weeks:* six male and 6 female rats were treated with DZ. *Group III; ordering effect:* six male and 6 female DZ-treated rats were injected with PK 11195, 10 mg/kg/IV, on Monday followed by flumazenil, 10 mg/kg, on Thursday. The following Monday each rat was injected IV with PK 11195 + flumazenil (10 mg of each) and with the vehicle, DMSO, on Thursday. This part of the study was designated as PK (1). A different group of 6 male and 6 female DZ-treated rats had the order of PK 11195 and flumazenil reversed. Flumazenil was administered on Monday, PK 11195 on Thursday, and the following Monday each rat was injected IV with PK 11195 + flumazenil on Monday and with DMSO on Thursday. This group was designated as PK (2).

Plasma and Brain Levels of DZ and its Metabolites

Three days after the 5th DZ capsule implant, blood was collected IV via a tail vein into EDTA tubes and weekly thereafter (or before the last precipitation) during the time spanned by the precipitated withdrawal studies for determining steady state plasma levels of DZ and its metabolites by HPLC as previously described (49). At the end of the study, the rats were euthanatized by decapitation, trunk blood was collected, and the brains were rapidly removed, frozen and stored at −70° until analyzed by HPLC (49). The pharmacokinetics of PK 11195 is reported elsewhere (82).

Plasma and Brain Levels of PK 11195 in DZ-Dependent Rats

Plasma and brain tissue were collected from different groups of age-matched DZ-dependent rats for 5 weeks (90 and 120 mg/capsule/week) female ($n = 6$) and male ($n = 7$) rats, respectively, than those used to test precipitated withdrawal (below). Trunk blood was collected and brains rapidly removed 5 min after the IV administration (tail vein) of PK 11195, 10 mg/kg, to rats that were euthanitized by decapitation. Plasma and brain tissue were harvested and stored at −70° centrigrade until removed for analysis (49) by HPLC as previously described (82).

Precipitated Withdrawal

The experiments started at approximately the same time each day (0900 h) and in repeated experiments, the rats were tested in the same order. The rat was placed in an observation arena and allowed to acclimate to its surroundings prior to obtaining baseline data. Without handling the rat, the antagonists and the vehicle were administered IV through a catheter (Jelco, 24 gauge) placed in the tail vein approximately 10 min before the start of baseline observations. The frequency of occurrence of each sign of precipitated withdrawal (PA) (respiratory rate was counted one time for each epoch) was identified by the same observer (frequently confirmed by a second observer) and recorded by a third observer in 5-min epochs for 10 min before and for 30 min after the injection of antagonists or vehicle. Both an average precipitated withdrawal score (PAS) and a maximum score (PAS_{MAX}) was calculated. The scored withdrawal signs included *convulsive phenomena*: clonic and tonic-clonic convulsions and twitches and jerks (isolated spasms of head, body or limbs); *motor signs*: head and body tremors, jumping, backing, turning; *motor dyskinesia*: writhing; some *aberrant behaviors* such as: a severely arched back, and vocalization; *autonomic sign*: respiratory rate (breaths/min counted with a stopwatch). The number of episodes of the above signs was summed (except respiration, which was counted once/epoch), weighted, added together for each epoch in order to generate the PAS as previously described (49,79). The PAS served as an indication of the intensity of the withdrawal syndrome. Several other withdrawal signs were also recorded but were not included in the PAS score. These withdrawal signs included chewing, digging, ear

twitches, blinking, head bobbing, hotfoot behavior, rigid tail, rigid walking, scratching, rearing, stretching of the body, wet dog shakes; and some postural and general activity signs such as flaccid, loss of righting reflex, curled posture, walking, standing, preening, and exploring. In order to normalize the data for between-subjects differences in baseline values, the mean preinjection values were subtracted from the postinjection values for each epoch for each rat prior to calculating the PAS. The average PAS (PAS_{AVG}) was calculated from the normalized area under the time action curves ($AUC_{0\text{-}30min}$).

Data Analysis

Statistical analyses included linear regression; paired and unpaired *t*-tests; one and two-way repeated measures analyses of variance (RM ANOVA); two-way ANOVAs with the appropriate post hoc comparisons. Sigma Stat software for Windows was used for these calculations, and a probability level of 0.05 or less was required for significance.

RESULTS

Body Weight

Age-matched male and female rats treated chronically for 5 weeks with silastic capsules containing DZ ($n = 6$) or empty capsules (controls, $n = 4$) showed a gender difference in weight gain (males > females), $F(1, 16)$ 229.5, $p < 0.001$; a significant between treatments effect, $F(1, 16)$ 42.3, $p < 0.001$, and the interaction, gender x treatments was significant, $F(1, 16)$ 23.2, $p < 0.002$ (2 way ANOVA of the average change in BW with gender and treatments taken as variables). Post-hoc multiple comparisons (Student-Newman-Keuls) revealed that male controls gained more BW (54.30 ± 2.7 g than female controls (10.36 ± 0.86g) $p < 0.05$. Further, male control rats gained more BW than either DZ-treated male (29.40 ± 2,34g), $p < 0.05$ or DZ-dependent female rats (6.65 ± 2.21g), $p < 0.05$. Whereas both male and female controls tended to gain more BW than DZ-dependent rats, this comparison was significant for male controls vs. male DZ-treated rats only (data not shown). Fig. 1 shows that just prior to PK 11195 administration, male DZ-treated and control rats had a greater increase in body weight than female DZ-treated and controls. The daily administration of PK 11195 abolished gender differences in BW gain in DZ-treated rats (Fig.1A) as well as in controls (Fig. 1B) after the first PK 11195 precipitation. The first PK 11195 precipitation produced the most dramatic decrease in BW gain in both DZ-treated and controls. There was a highly significant gender difference (males>females) in control rats, $F(1,12)$ 30.4, $p < 0.018$ (2-way RM ANOVA of BW gain (gender and days of PK 11195 treatment taken as variables). In both DZ-treated and control rats, the effects of PK 11195 were significantly related to the day of treatment, $F(4, 36)$ 16.10, $p < 0.0001$ (DZ treated); $F(4, 12)$ 44.2, $p < 0.001$(controls) and there was a gender x days interaction, $F(4,36)$ 14.53, $p < 0.0001$(DZ-treated); $F(4, 12)$ 22.4, $p < 0.001$ (controls). DZ-dependent male rats gained significantly less BW than male controls, $F(1, 32)$ 52.95, $p < 0.0001$, whereas PK 11195 induced a time-related decrease in BW gain in both controls and DZ-dependent male rats, $F(4,32)$ 96.24, $p < 0.001$ (2-way RM ANOVA with treatment and days taken as variables). The daily administration of PK 11195 to male and female DZ-dependent and control rats significantly decreased BW gain in all groups relative to the prePK 11195 weights ($p < 0.05$, Student-Newman-Keuls Method) (Fig. 1A and B).

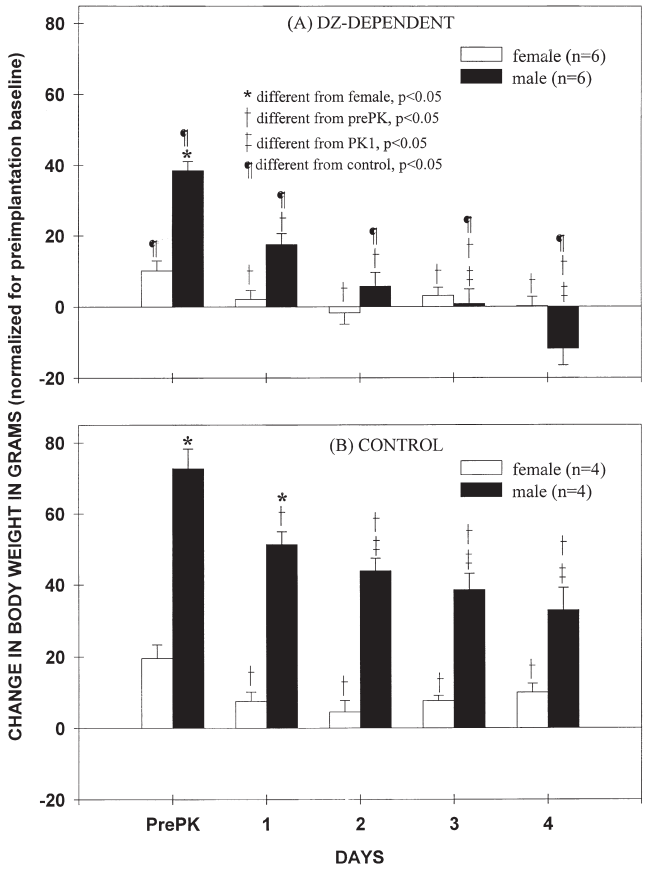

FIG. 1. Change in BW induced by the daily administration of PK 11195 (10 mg/kg/IV) to male and female rats chronically exposed to DZ, 120 and 90 mg/capsule/week, male and female respectively, (A) and in male and female controls (1 empty capsule/week) (B). Each value, normalized for the preimplant baseline, represents the mean change in BW weight + SE for the number of rats indicated.

Fig. 2 illustrates the weekly change in BW in DZ-dependent rats prior to PK 11195 and after the weekly administration of PK 11195. Gender differences in BW gain were seen (M>F) for weeks 1, 3, 4, and 5 pre-PK and weeks 4 and 5 post-PK. The greatest inhibition of BW gain occurred after the first dose of PK 11195 (week 1) in either female or male rats. Thereafter no significant effect of PK 11195 was observed on BW gain in either sex with the exception of week 4 in male rats.

Plasma and Brain Levels of DZ and its Metabolites

The mean stabilization plasma levels of DZ and its metabolites showed no significant gender difference in rats treated chronically with DZ for 5 weeks. Daily injections of PK 11195 did not significantly affect plasma levels of BZs as can be seen in Table 1. Table 2 shows plasma and brain levels of DZ and its metabolites in rats (different rats from those in Table 1) subjected to the weekly administration of PK 11195 as well as in rats administered PK 11195 and flumazenil in reversed order (each order in different groups of rats). As can be seen in Tables 1 and 2, the parent compound, DZ, is the major drug detected in plasma or brain. Although there was a trend to-

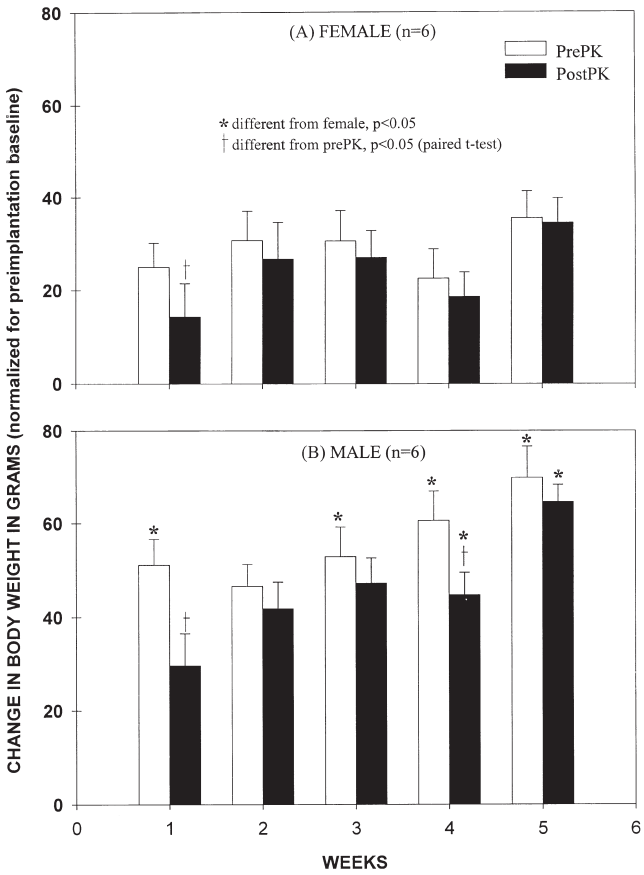

FIG. 2. Change in BW induced by the weekly administration of PK 11195 to DZ- dependent (see legend to Fig. 1) female (A) and male rats (B). Open bars represent the prePK BW change from preimplantation baseline and solid bars represent the post-PK change from preimplantation baseline. Each value represents the mean + SE for 6 rats.

ward higher levels of DZ in female than in male rats in each treatment group shown in Table 2, no significant gender differences were observed. On the other hand, brain levels of ND were significantly higher in female than in male rats challenged with PK 11195 and flumazenil administered in different order, $F(1, 23)$ 14.5; $p < 0.0025$; 2-way ANOVA.

Plasma and Brain Levels of PK 11195 in DZ-Dependent Rats

Plasma and brain levels of PK 11195 could not be done in the same group of rats in which precipitated withdrawal was measured. However, in separate groups of age-matched male and female DZ-dependent rats subjected to identical PK 11195-induced withdrawal as in the present study, PK 11195 levels were measured in plasma and brain tissue harvested at the time of peak withdrawal (5 min post-PK 11195). The data showed no statistically significant gender difference in either brain or plasma levels of PK 11195, although the levels tended to be higher in males in both plasma and brain tissue and both sexes showed higher levels in brain than in plasma (Table 3).

Effect of the Daily Administration of PK 11195 on the PAS in DZ-Dependent Rats

During the repeated daily administration of PK 11195 study, female DZ-dependent rats had fewer clonic convulsions on days 2 through 5 than on day 1 as did males on days 3 and 4. No trend was seen with regard to tonic-clonic convulsions. Twitches and jerks declined steadily in both males and females through day 3, a tendency that was partially reversed by days 4 and 5. These trends were not significant, however (Table 4). No convulsive activity was observed in the controls.

The PK 11195-induced PAS in these DZ-dependent rats usually reached a maximum effect within 5 min (PAS_{MAX}). Rapid tolerance to PK 11195 developed by day 2 in either sex with regard to the PAS_{MAX} (Fig.3A). Interestingly, female DZ-dependent rats began to lose tolerance by day 3, whereas the PAS_{MAX} induced in male rats remained depressed through day 5. This was a significant gender difference, (two-way RM ANOVA with gender and days taken as factors) $F(1, 40)$ 6.2, $p < 0.05$, with no significant between days or gender x days interaction. No effect of PK 11195 on the PAS_{MAX} was seen in control rats of either sex, nor were gender differences observed across the 5-day test. The PAS_{MAX} induced by PK 11195 in male DZ-treated rats was significantly greater than in control rats, $F(1, 32)$ 78.09, $p < 0.0001$, as was the between days, $F(4, 32)$ 3.67, $p < 0.01$, and the interaction, treatment x days, $F(4, 32)$ 4.45, $p < 0.01$, two-way RM ANOVA). Post-hoc multiple comparison procedures (Student-Newman-Keuls) showed that the PAS_{MAX} induced on days 2, 3, 4, and 5 in DZ-treated male rats was significantly less than on day 1. Similarly, the PAS_{MAX} induced in female DZ-dependent rats was significantly greater than in female control rats $F(1, 32)$ 16.74, $p < 0.005$. There was no significant between days dif-

TABLE 1

PLASMA LEVELS* OF DZ AND ITS METABOLITES IN MALE AND FEMALE DZ-DEPENDENT RATS ON DAY 1 PRIOR TO THE DAILY INJECTION OF PK 11195 (10 mg/kg) FOR 5 DAYS AND ON DAY 5 AFTER THE LAST INJECTION OF PK11195

	Males		Females	
Drug	Day 1	Day 5	Day 1	Day 5
DZ	2.05 ± 0.37	1.87 ± 0.57	1.44 ± 0.41	1.50 ± 0.12
ND	0.46 ± 0.22	0.04 ± 0.04	0.18 ± 0.07	0.13 ± 0.06
TM	0.47 ± 0.21	0	0.07 ± 0.05	0
OX	0.59 ± 0.45	0.59 ± 0.51	0.05 ± 0.03	0.19 ± 0.13

*Values are the mean (μg/ml of plasma) ± SEM (n = rats/sex).

TABLE 2

PLASMA AND BRAIN LEVELS OF DZ AND ITS METABOLITES IN *RATS MADE DEPENDENT ON DZ RELEASED FROM SILASTIC CAPSULES AND SUBJECTED TO BZ ANTAGONIST-INDUCED WITHDRAWAL AS INDICATED

	Females		Males	
Drug	Plasma (μg/ml)	Brain (μg/g)	Plasma (μg/ml)	Brain (μg/g)
†WEEKLY PK PRECIPTIATED WITHDRAWAL				
DZ	2.02 ± 0.43	4.90 ± 2.39	1.32 ± 0.60	1.64 ± 0.08
ND	0.12 ± 0.06	0.12 ± 0.06	0.44 ± 0.32	0.17 ± 0.11
TM	0.05 ± 0.05	0.32 ± 0.26	0.00 ± 0.00	0.24 ± 0.15
OX	0.08 ± 0.08	0.20 ± 0.07	0.00 ± 0.00	0.23 ± 0.08
‡PK → FLU				
DZ	2.12 ± 0.75	2.20 ± 0.53	0.63 ± 0.04	1.33 ± 0.22
ND	0.16 ± 0.06	0.57 ± 0.14	0.24 ± 0.07	0.02 ± 0.02
TM	0.04 ± 0.04	0.37 ± 0.17	0.05 ± 0.05	0.92 ± 0.35
OX	0.08 ± 0.08	0.12 ± 0.08	0.05 ± 0.05	0.20 ± 0.11
§FLU → PK				
DZ	3.29 ± 1.74	1.19 ± 0.11	0.73 ± 0.12	1.26 ± 0.08
ND	0.12 ± 0.06	0.32 ± 0.10	0.05 ± 0.05	0.14 ± 0.09
TM	0.14 ± 0.09	1.22 ± 0.10	0.00 ± 0.00	0.56 ± 0.36
OX	0.13 ± 0.09	0.34 ± 0.09	0.11 ± 0.11	0.08 ± 0.05

*All rats (n = 6/sex/group) were treated with weekly implantation of DZ (90 and 120 mg for females and males, respectively). The precipitation studies were initiated after the 5th implantation. After completion of the precipitated withdrawal, rats were decapitated and trunk blood and brain tissue were obtained for determination of plasma and brain levels of DZ and its metabolites. Values are the mean ± SEM of 6 rats in each group.

†Rats were subjected to PK-precipitated withdrawal at weekly intervals for 5 weeks.

‡Rats were administered PK on Monday followed by FLU on Thursday of same week and then mixture of FLU and PK on Monday followed by DMSO on Thursday of the following week.

§Rats were administered FLU on Monday followed by PK on Thursday of same week and then mixture of FLU and PK on Monday followed by DMSO on Thursday of the folllowing week.

ference in naive vs. DZ-treated female rats nor was there a significant days x treatment interaction.

Tolerance also developed to the effect of the daily administration of PK 11195 on respiration, one of the signs comprising the PAS_{MAX}. The maximum effect on respiratory rate (RR_{MAX}) declined markedly in both male and female DZ-dependent rats by day 2. Tolerance persisted through day 5 in both sexes. A two-way RM ANOVA of the RR_{MAX} in DZ-dependent rats with gender and days taken as factors showed no gender difference nor was there a significant gender x day interaction. There was, however, a highly significant between-days decrease in RR_{MAX}, $F(4, 40)$ 6.01, $p < 0.01$. The RR_{MAX} was significantly less on days 2, 3, 4, and 5 than on day 1 in DZ-dependent rats (Student-Newman-Keuls, $p < 0.05$). No tolerance developed to PK 11195 in male and female controls nor were there gender differences except on day 4 when female controls showed a higher RR_{MAX} than males (unpaired t-test). (Fig. 3B).

Effect of the Weekly Administration of PK 11195 on the PAS in DZ-Dependent Rats

Although there was a trend in DZ-dependent male rats administered PK 11195, 10 mg/kg, at weekly intervals for 5 weeks for twitches and jerks to increase with time, no significant between weeks difference was observed in any convulsive sign. Female DZ-dependent rats did, however, show a between weeks difference in twitches and jerks, RM ANOVA on ranks, $\chi^2 = 12$ with 4 df $p < 0.02$, and a significant positive regression of twitches & jerks with increasing

TABLE 3

LEVELS OF *PK 11195 IN †DIAZEPAM DEPENDENT RATS (μg/ml or μg/g)

Gender	‡Plasma	‡Brain	Brain/Plasma
Females (n = 6)	3.31 ± 1.38	15.5 ± 12.42	2.12 ± 1.25
Males (n = 7)	4.56 ± 1.04	22.4 ± 8.12	4.52 ± 0.89

*PK 11195 Dose: 10 mg/kg/IV

†Diazepam-dependent rats, Dose: (Females = 90 mg/cap/week: Males = 120 mg/cap/week

‡Plasma and brain tissue were collected at 5 min post PK 11195.

TABLE 4

THE EFFECT OF THE REPEATED ADMINISTRATION OF PK (10MG/KG/IV) FOR 5 DAYS ON CONVULSIVE SIGNS IN DZ-DEPENDENT MALE AND FEMALE RATS

	Male Rats			Female Rats		
Day	T&J	CC	T-CC	T&J	CC	T-CC
1	15* (4/6)†	3 (2/6)	0 (0/6)	9 (3/6)	6 (3/6)	1 (1/6)
2	3 (3/6)	3 (2/6)	1 (1/6)	7 (3/6)	0 (0/6)	0 (0/6)
3	1 (1/6)	1 (1/6)	0 (0/6)	3 (2/6)	5 (1/6)	1 (1/6)
4	3 (3/6)	0 (0/6)	0 (0/6)	10 (3/6)	1 (1/6)	1 (1/6)
5	10 (4/6)	4 (1/6)	0 (0/6)	5 (3/6)	5 (2/6)	0 (0/6)

*Sum of convulsive signs observed in the animals in the group

†Number of rats that exhibited convulsive signs/number of rats in group

T&J = Twitches and Jerks

CC = Clonic Convulsions

T-CC = Tonic-Clonic Convulsions

weeks, F (1, 28) 4.76, $p < 0.05$, but no change in clonic or tonic-clonic seizures. There was also a tendency for the number of rats showing twitches and & jerks to increase with weeks in both male and female rats (Table 5). In contrast to its daily administration (5 days), the weekly (5 weeks) administration of PK 11195, 10 mg/kg/IV, to male and female DZ-dependent rats did not produce tolerance to its effects on the PAS_{MAX} in either sex (Fig. 4A). Further, tolerance did not develop to the ability of PK 11195 to induce tachypnea (shown as RR_{MAX} a sign included in the PAS) (Fig. 4B).

Effect of the Order of Administration of PK 11195 and Flumazenil on the PAS in DZ-Dependent Rats

No significant ordering effect was observed for any of the PK 11195-induced convulsive signs (clonic convulsions, tonic-clonic convulsions, and twitches and jerks) when PK 11195 (10 mg/kg) was administered on Monday and flumazenil on Thursday to groups of 6 female and 6 male rats [designated as PK(1)] and the effects compared with different groups of 6 female and 6 male rats that had the order of PK 11195 and flumazenil administration reversed [designated as PK(2)] (see Table 6). An interesting previously observed phenomenon is the fact that the rats that had convulsions after PK 11195 were different rats from those that had convulsions after flumazenil. PK 11195 + flumazenil produced a less than additive effect for cc in both male and females (Table 5).

No significant ordering effect was observed in the PAS_{MAX} induced by PK 11195, flumazenil, or the combination of PK 11195 + flumazenil in either female or male rats (Fig. 5). DMSO did not produce a significant PAS_{MAX} in DZ-dependent rats; and PK 11195 did not produce a significant PAS in the empty capsule control rats. Similarly, flumazenil has been repeatedly shown to produce no significant PAS in male and female control rats (data not shown). Flumazenil induced a significantly greater PAS_{MAX} in female than in male rats in the PK (1) group, a tendency which was also seen in females in the PK(2) group. In contrast to flumazenil, PK 11195 tended to induce a higher PAS_{MAX}in both the PK(1) and PK(2) groups in male than females in these groups. Note that with regard to the PAS_{MAX}, PK 11195 + flumazenil produced a less-than-additive effect in both sexes. No ordering effect was seen for the sequence of flumazenil and PK 11195 administration on any of the signs comprising the PAS. Two of these signs (respiratory rate and vocalization) that showed gender differences, but no ordering effect, are shown in Figs. 6 and 7] respectively. A greater degree of tachypnea was induced by PK 11195 in female than in male rats in the PK (1) group, $p < 0.05$ (unpaired t-test) and there was a tendency in this direction for the PK (2) group. In contrast, flumazenil tended to produce more tachypnea in male than in female rats regardless of the sequence of administration. Gender-related differences were also seen with regard to vocalization. Female rats vocalized significantly more than male rats after flumazenil in the PK(2) group ($p < 0.05$, unpaired t-test) and tended to vocalize more after any IV injection than male rats in either the PK(1) or the PK(2) groups (Fig. 7).

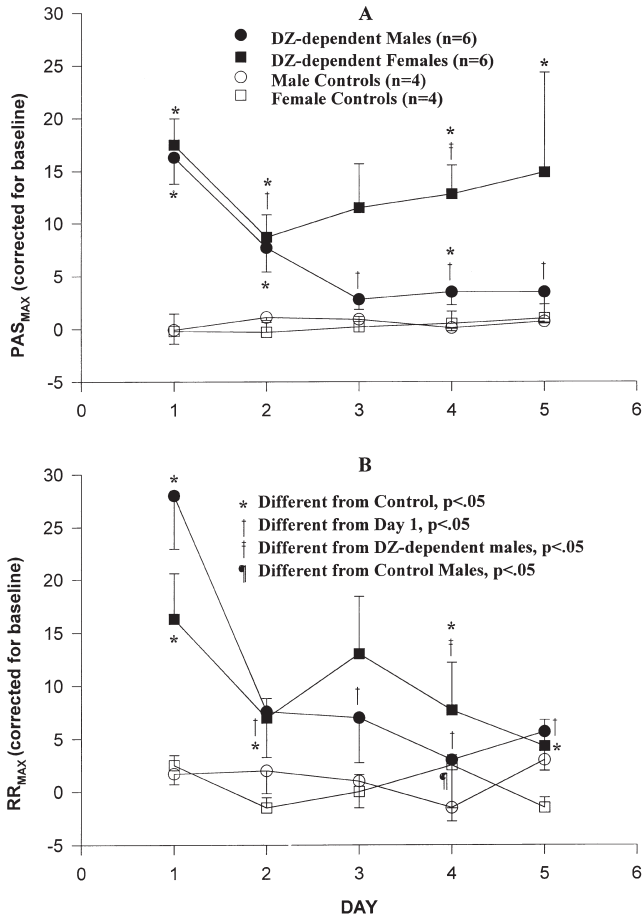

FIG. 3. Time course of the development of tolerance to the daily administration of PK 11195 (10 mg/kg/IV) for 5 days in rats treated chronically with DZ, 90 mg/kg/week (females); 120 mg/kg/week (males), or with empty capsules (controls) (A). (B) Shows the time course of the development of tolerance to PK 111955-induced increase in RR_{MAX} when the antagonist is administered daily. Each point represents the mean for the number of DZ-dependent and control rats indicated. + SE.

DISCUSSION

The gender differences observed in BW gain during the 5-week stabilization period (males>females) is consistent with the observation that the estrous cycle influences feeding patterns in female rats (43) and with the finding that BW is increased by castration in females, whereas it is decreased in

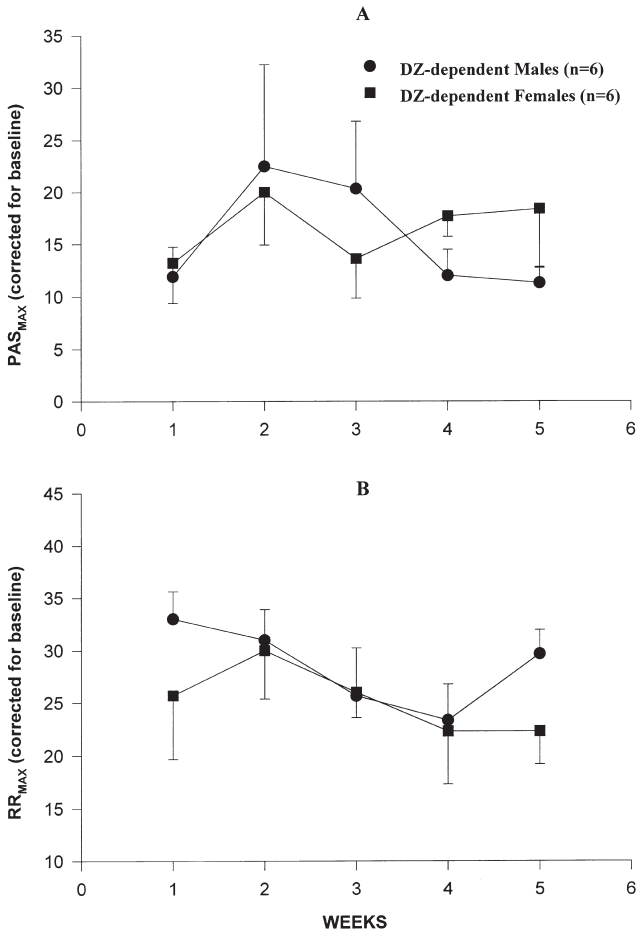

FIG. 4. The weekly PAS_{MAX} induced by PK 11195 in DZ-treated rats (5 weeks prior to precipitated withdrawal) (A) and the weekly RR_{MAX} (B). Each point represents the mean + or − SE for the number of rats indicated.

male rats (67). Evidence suggests that the paraventricular nucleus of the hypothalamus mediates the estrogen-induced enhancement of the satiety effect of cholecystokinin (6,7,19). The CBR within the brainstem have been implicated in BZ-enhanced food consumption (30,58), an effect antagonized by flumazenil (46). Not all data are in agreement concerning the effects of BZs on feeding behavior (See 11,28, for review). It has been argued (28) that most of the studies showing BZ hyperphagia measured food intake after acute treatment and that rats treated chronically with BZs eat less in the long run and gain less weight than vehicle-treated controls. The present study and previous reports (33,34,63) show that rats treated chronically with DZ gain less weight than controls and that in either DZ-treated or controls, male rats gain more BW than females. A similar observation was also made in mice (44) in which castration as well as the administration of testosterone or estrogen abolished the effect of chronic DZ treatment on BW in males but not in females. Further, there is considerable evidence that many actions of BZs are mediated through the PBR resulting in a variety of gender-related effects although little is known about the effect of PK 11195 on feeding behavior or the pharmacodynamics of PK 11195-induced weight loss. In this regard, chronic exposure to DZ

TABLE 5

THE EFFECT OF THE REPEATED ADMINISTRATION OF PK (10MG/KG/IV) FOR 5 WEEKS ON CONVULSIVE SIGNS IN DZ-DEPENDENT MALE AND FEMALE RATS

	Male Rats			Female Rats		
Week	T&J	CC	T-CC	T&J	CC	T-CC
1	8* (3/6)†	5 (2/6)	1 (1/6)	6 (3/6)	5 (2/6)	0 (0/6)
2	19 (3/6)	0 (0/6)	0 (0/6)	17 (4/6)	2 (1/6)	2 (1/6)
3	20 (4/6)	3 (1/6)	1 (1/6)	18 (5/6)	0 (0/6)	0 (0/6)
4	19 (5/6)	2 (1/6)	0 (0/6)	90 (6/6)	2 (1/6)	0 (0/6)
5	30 (6/6)	0 (0/6)	0 (0/6)	33 (6/6)	0 (0/6)	0 (0/6)

*Sum of convulsive signs observed in the animals in the group
†Number of rats that exhibited convulsive signs/number of rats in group
T& J = Twitches and Jerks
CC = Clonic Convulsions
T-CC = Tonic-Clonic Convulsions

caused a down-regulation of the testicular PBR and a significant fall in plasma levels of testosterone (9), whereas ovarian PBR were upregulated with no accompanying change in serum levels of progesterone and estradiol (85). The PBR agonist, 4' chlordiazepam, produced dose-related hyperphagia, an effect prevented by PK 11195 and picrotoxin but not by the CBR antagonist flumazenil (64). In the present study, the administration of PK 11195 (10 mg/kg) to either control or DZ-treated rats at daily intervals induced a linear decrease in BW in males but not in females. When PK 11195 was administered at weekly intervals to DZ-treated rats, the greatest decrease in BW was seen with the first injection of PK 11195 and thereafter the inhibition in BW gain decreased with time until at week 5, PK 11195 produced little effect on BW gain. The fact that PK 11195 diminished BW gain in both controls and DZ-treated rats during daily PK 11195 administration suggests that BW gain is not mediated through the PBR. There is, however, another possible explanation. The PBR could be indirectly responsible for these BW effects in DZ-treated as well as in control rats through changes induced in steriodogenesis and subsequent modulation of $GABA_A$ receptors that may preferentially recognize either neurosteroids or BZs (64). These data taken together are consistent with the concept that the hormonal milieu modifies BW gain through poorly understood mechanisms.

The present study clearly demonstrates that the daily administration of PK 11195 (10 mg/kg IV) to DZ-dependent rats reduces its ability to induce a precipitated withdrawal syndrome by day 2 in both male and female rats. There is, however, a marked gender difference in this effect. By day 3, PK 11195 produced a higher PAS_{MAX} in female rats than on day 2, a pattern that continued until the PAS_{MAX} was 80% of day 1 by day 5. In contrast, the PAS_{MAX} in males on day 3 was markedly reduced to a point where almost complete tolerance to the antagonistic actions of PK 11195 persisted through day 5. Whereas some tolerance appeared to develop to the ability of PK 11195 to induce twitches and jerks in both sexes, no pattern of tolerance was seen with regard to clonic and tonic-clonic convulsions. Further, PK 11195 (10 mg/kg) had little or no intrinsic effect on the PAS in controls.

These findings emphasize the fact that dose-response curves for PK 11195 cannot be reliably generated for some precipitated withdrawal responses in either male or female DZ-dependent rats when the drug is administered on a daily

TABLE 6
ORDERING EFFECT OF PK-FLU ADMINISTRATION ON CONVULSIVE SIGNS

	Pk → FLU*			FLU → PK†		
Sign	PK	FLU	PK + FLU	PK	FLU	PK + FLU
Females						
CC	4‡ (3/6)¶	2 (1/6)**	3 (2/6)**	2 (1/6)	4 (3/6)**	2 (1/6)**
TCC	0 (0/6)	0 (0/6)	1 (1/6)**	0 (0/6)	0 (0/6)	0 (0/6)
T & J	4 (3/6)	13 (5/6)	17 (5/6)	14 (4/6)	10 (2/6)	16 (6/6)
Males						
CC	3 (1/6)	0 (0/6)	2 (1/6)**	2 (1/6)	2 (2/6)**	2 (2/6)
TCC	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
T & J	8 (4/6)	5 (5/6)	8 (3/6)	8 (4/6)	2 (2/6)	8 (3/6)

*PK administered 3 days before FLU.
†PK administered 3 days after FLU.
‡number of convulsive episodes
¶number of rats that had convulsive episodes/number of rats in group
**different rats from those that had CC with PK (rats that convulsed with FLU did not convulse with PK)

basis. Because PK 11195 is administered at repeated intervals to the same subject to assess the role the PBR play in a variety of physiologic processes such as: BZ dependence (50,51,70,80); steroidogenesis (40); immunomodulatory actions of BZs (57); ammonia toxicity in hepatic encephalopathy (32), this is an important issue to consider in experimental design. The finding that, in contrast to daily dosing, tolerance did not develop to the antagonistic effects of PK 11195 when it was administered at weekly intervals to either male or female rats is reminiscent of previous findings with the CBR antagonist, flumazenil, in the diazepam-dependent dog, rat, and

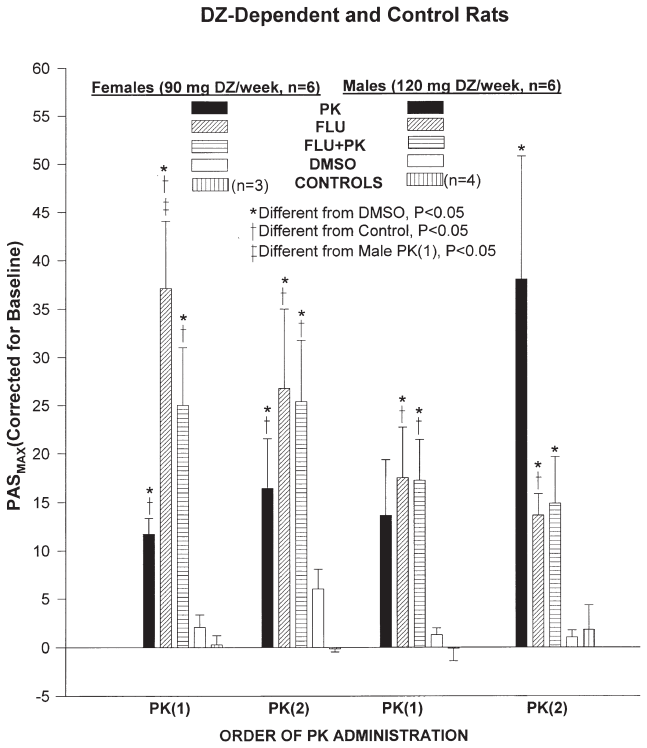

FIG. 5. The PAS_{MAX} induced by PK 11195 (PK), flumazenil (FLU), PK + FLU and DMSO in DZ-dependent rats (5 weeks) when PK was administered on Monday and FLU on Thursday. The following Monday, PK + FLU was administered followed by DMSO on Thursday. This sequence is indicated as PK(1). The order of PK and FLU administration was reversed in the second group of rats and is designated as PK(2). Control rats were injected with PK 11195 only. DMSO was the solvent for PK 1195 and flumazenil. Only DZ-dependent rats were injected with DMSO. Each bar represents the mean + SE for the number of rats indicated.

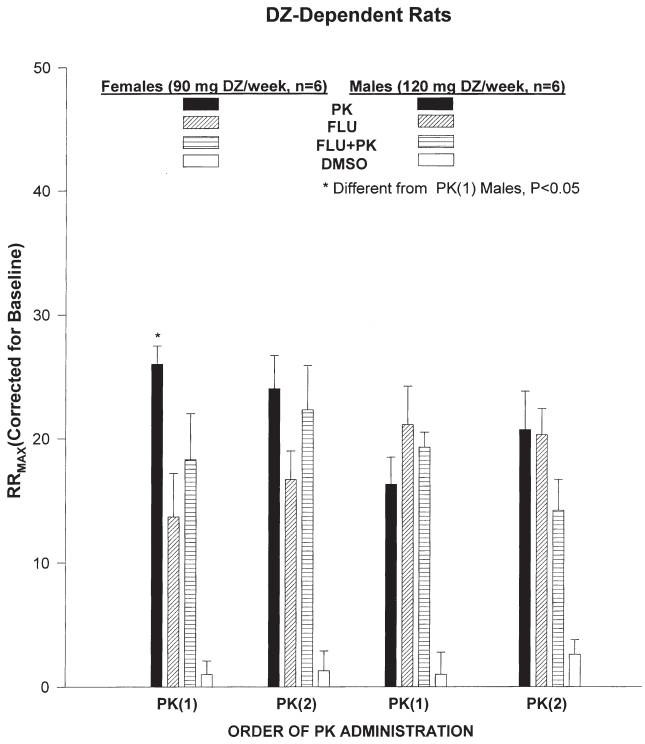

FIG. 6. The RR_{MAX} (one of the signs included in the PAS_{MAX}) induced by PK 11195 (PK), flumazenil (FLU), FLU + PK, and DMSO. Refer to Fig. 5 for the order of PK, FLU, PK + FLU and DMSO administration. Each value represents the mean + S.E. for the number of rats indicated.

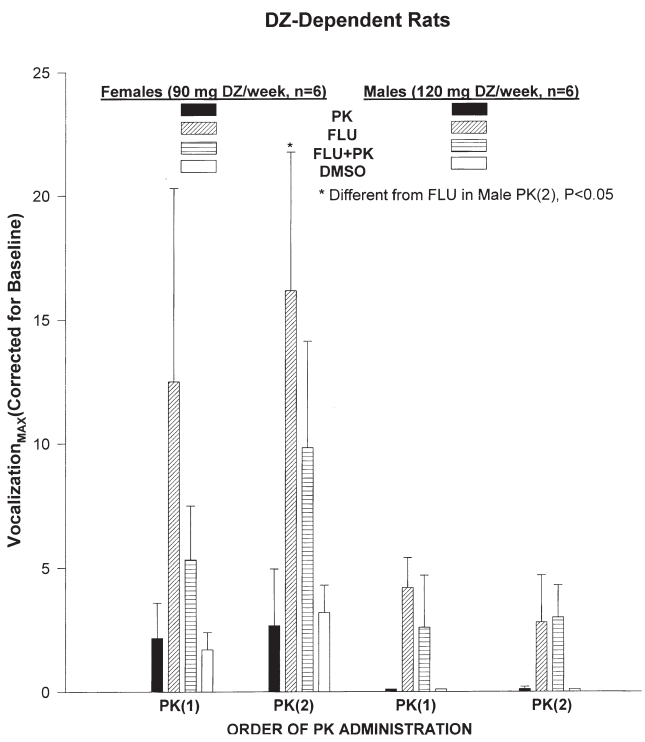

FIG. 7. Vocalization$_{MAX}$ (one of the signs included in the PAS$_{MAX}$) induced by PK 11195 (PK), flumazenil (FLU), FLU + PK, and DMSO in DZ-dependent rats. Refer to Fig. 5 for the order of PK, FLU, PK + FLU and DMSO administration. Each value represents the mean + S.E. for the number of rats indicated.

squirrel monkey (46,47–49,68,70). Further, as the time course of diazepam treatment lengthened, the level of dependence increased with regard to some (convulsive) but not all, signs of the PK 11195-induced PAS. This is similar to the observations showing that DZ dependence, as measured by the flumazenil-induced precipitated withdrawal response, increases with time (44,48,93). The involvement of PBR in seizure activity is supported by previous studies (55) showing that the PBR are involved in the pathogenesis of epileptic seizures in seizure prone El mice.

The underlying neurochemical basis for the observed gender differences in the development of tolerance to the antagonist effects of PK 11195 in DZ-dependent rats is not clear. Tolerance can be classified as either pharmacodynamic (functional) or pharmacokinetic (dispositional). Pharmacodynamic tolerance results from a decreased sensitivity to the effects of a drug (receptor down-regulation or desensitization). Pharmacodynamic tolerance can also arise by homeostatic changes downstream that counteract the initial effects of the drug. Pharmacokinetic tolerance arises from changes in absorption, distribution, metabolism or excretion of the drug resulting in lowered concentrations of the drug at the receptor sites (10,14).

Evidence for Pharmacodynamic Tolerance. There is some evidence that in DZ-dependent male rats the PBR are upregulated in the heart and cerebral cortex (84), whereas others find no change in receptor numbers (20). To the best of our knowledge, it is not known whether the density of the PBR is altered in the brain of the DZ-dependent female rat. It is known that gonadal hormones can influence PBR density. There is a large body of evidence indicating that the PBR are the critical factor in the rate-limiting step of steroidogenesis; that the PBR as well as the CBR are tonically and physically regulated by neural and hormonal mechanisms, both in the periphery and within the brain of either sex. Stress can modify both CBR and PBR and DZ can alleviate some of these stress-induced changes (5,13,15,24,29). All rats in the current experiments were subjected to repeated handling for 5 weeks prior to precipitation studies at which time they showed no obvious signs of stress when handled. Antagonists were injected remotely via an indwelling tail vein catheter, a procedure that did not appear to be stressful except for the withdrawal effects of the drugs. It has been demonstrated that precipitated DZ withdrawal did not increase anxiety levels (plus-maze) in either male or female rats (71). On the other hand, it has been shown repeatedly in humans and in animals by assessing the overall signs of either precipitated or non-precipitated withdrawal in BZ-dependent subjects that the condition is stressful (18,48,50,93). It is not known what influence gender and brain region exert on neurosteroid modulation of the PBR in DZ-dependent rats. Within the CBR/GABA$_A$ complex, on the other hand, it has been reported that GABA$_A$ receptors do not vary over the rat estrous cycle in either drug naive rats, or in rats acutely and chronically treated with DZ. Further, significant correlations are not seen between circulating levels of estradiol or progesterone and CBR binding parameters in cycling female rats. Within this system, there are, however, marked variations in brain region and gender response to the hormonal milieu with regard to the potentiation of GABA responses by the neuroactive steriod derivatives of progesterone, testosterone and glucocorticoids (67,87,89). It also seems likely that there are regional gender variations in the hormonal milieu that in turn regulate PBR density in the DZ-dependent rat brain that could be modulated rapidly and differently by the repeated administration of PK 11195 (4,8,13) and contribute to differences in the degree of tolerance development to PK 11195. Steroids secreted by the gonads and adrenals are involved in regulating the interplay between the brain and these organs in ways other than as feedback messengers. These actions at the cellular level, possibly through specific cytosolic receptors, lead to such actions as regulation of neurotransmitter or second messenger systems, and alterations in nuclear gene expression (39). In this regard the GLUTAMATergic system has been shown to modulate BZ tolerance and discontinuation (38).

To summarize, although no measures of receptor density, affinity, desensitization, or of neurosteroid and neurotransmitter release were made, it seems highly likely that the hormonal milieu played a major role in the tolerance observed in the daily PK 11195 precipitated withdrawal experiments.

Evidence for Pharmacokinetic Tolerance. Another factor which should be discussed is the possible influence of pharmacokinetics. These studies were conducted when the levels of DZ and its metabolites were at steady state. Therefore, it seems likely that the critical drug to examine with regard to the tolerance that developed to the PK 11195-induced PAS$_{MAX}$ is the pharmacokinetics of PK 11195 itself. This does not rule out the possibility that an interaction between PK 11195 and DZ and/or its metabolites may also play a role in the gender differences observed. It is fairly well-established that in BZ-naive subjects, BZs are metabolized faster in males than in female rats (65,72,83). The plasma clearance of DZ is greater in male Wistar (7) than in female Fisher rats (73), which agrees with observations in BZ-naïve humans (26,27).

In comparison to females, male rats have been reported to have lower brain levels of DZ after continuous exposure to silastic implants containing 90 mg of DZ/capsule (88). Plasma and brain levels of PK 11195 could not be determined in the same animals that were precipitated repeatedly with PK 11195 on either a daily or weekly basis. However, in parallel studies (82), the pharmacokinetics of acutely administered PK 11195 (5, 10, and 20 mg/kg IV) to DZ-naive male and female rats showed no relationship to dose or gender for either the half-life for distribution (~0.14 h) or for elimination (~5.4 h). On the other hand, the total plasma clearance increased with increasing dose (23 to 42 ml/min/kg). Gender differences were seen for the 10 mg/kg dose of PK 11195 (the dose used in the present studies) in the total clearance which was significantly faster for males than for females. The volume of distribution was large (9 to 24 L/kg) suggesting an extensive distribution outside plasma and tended to be higher for all doses in males than in female rats. The limited data collected in these studies indicates that PK 11195 accumulates in the brain with a brain/plasma ratio of ~3 without relation to dose or gender. Although a complete pharmacokinetic profile of PK 11195 has not been done in DZ-dependent male and female rats, plasma and brain levels were measured at 5 min post-PK11195 in different groups of rats with identical DZ and PK 11195 dosing to that used in the current study for day 1 of either the daily or weekly PK 11195 precipitated withdrawal. Significant gender differences were not seen in either brain or plasma levels of PK 11195 (82). Further, in the present studies, gender differences were not seen in the PAS_{MAX} (5 min) after a single dose of PK 11195. A different picture emerged, however, when the rats were challenged with multiple doses of PK 11195 given in daily intervals where the PAS was higher in female than in male rats at days 3 to 5. There is a lack of data on brain levels of PK 11195 after multiple dose treatment. It is possible that accumulation of PK 11195 in the brain is different in male and female DZ-dependent rats.

In summary, although it seems likely that gender difference in the pharmacokinetics of PK 11195 could have contributed to the gender differences in the degree of tolerance to its effect on the PAS, the lack of pharmacokinetic data for PK 11195 during its repeated administration (each day) to DZ-dependent rats does not allow us to determinate to what extent pharmacokinetics influenced the observed tolerance.

Changes in Convulsive Signs During Weekly PK 11195 Precipitated Withdrawal

Both flumazenil and PK 11195 induced convulsive signs in male and female rats that had been administered DZ chronically. Twitches and jerks tended to increase across time with the weekly but not with the daily administration of PK 11195 in both sexes. This is in line with previous observations in female DZ-dependent dogs and male rats and baboons precipitated with flumazenil (33,44,90,93). This increase in convulsive activity with time may be related to a flumazenil-induced increase in the firing rate of substantia nigra pars reticulata neurons of diazepam-treated rats which, in turn, may help propagate a flumazenil-induced seizure from another brain structure (90,91). In the present study, only females showed a significant positive regression of twitches and jerks with weeks. Others have shown that in DZ-dependent mice, males had a lower incidence of flumazenil-induced seizures than females. Castration significantly increased seizure activity in male but not female mice, an effect reduced by testosterone or estrogen in male but not in females. This suggests that gender differences in DZ dependence is modulated by the action of hormones, that testosterone plays a relevant role (59) and confirms previous findings that both CBR and PBR are involved in the dependence producing properties of DZ (50,51,70,78,81).

Effect of the Order of Flumazenil and PK 11195 Administration(Group III Rats). The order of flumazenil and PK 11195 administration did not alter the PAS_{MAX}. The two antagonists may be administered to the same rat of either sex without altering the PAS if the doses are separated by as much as 3 days, the shortest time period tested. Further, no ordering effect was observed in plasma levels of either DZ or its metabolites in either sex. Interestingly, male or female rats in either the PK(1) or the PK(2) groups that had clonic or tonic-clonic convulsions with flumazeninl or with PK 11195 + flumazenil were different rats from those that had convulsions with PK 11195. This agrees with previous observations in our laboratory and supports the concept that flumazenil- and PK 11195-induced convulsions are mediated through different receptors. It should be pointed out that this observation did not hold for twitches and jerks.

Although no ordering effect was produced in this study, gender differences were observed in some measures: 1) flumazenil induced a higher PAS_{MAX} in female than in male rats in the PK (1) group, and a tendency in this direction was also observed in the PK (2) group. 2) Female rats had a higher RR_{MAX} after PK 11195 than male rats in the PK (1) group with a tendency in this direction for the PK (2) group. 3) Female DZ-dependent rats tended to vocalize more than male rats after flumazenil, PK 11195 or their combination in either the PK(1) or the PK(2) group and significantly so after flumazenil in the PK(2) group. Vocalization is probably a response to perceived pain. In this regard, central morphine analgesia is reported to be greater in male than in female rats (36) and women are reported to have lower pain thresholds and lower pain tolerance than men (77).

The combination of PK 11195 + flumazenil produced a less-than-additive effect on the PAS_{MAX} and on most signs comprising it. This could be partially explained by the fact that PK 11195 produces a less robust PAS than flumazenil. This suggests that when the 2 drugs are combined, they interact with each other in some way. One possibility is through different effects on the GLUTAMergic and GABAergic systems. Striatal neuronal activity has been shown to be increased by glutamate and GABA can modulate both basal and glumate-evoked effects (37). Further, NMDA receptor subunit proteins are upregulated in the cerebral cortex of male rats during DZ withdrawal (74) and NMDA receptor antagonists have been shown to suppress withdrawal signs induced by discontinuation of long-term DZ treatment in male rats (75).

Taken together, these data provide further evidence that both central and peripheral receptors are involved in the dependence-producing properties of DZ; that PK 11195 can be repeatedly administered at weekly (but not at daily intervals) to the same DZ-dependent rats without altering the precipitated withdrawal response; that there is no significant ordering effect on the PAS_{MAX} that results from the administration of PK 11195 and flumazenil to the same rat when the dosing interval is separated by 3 days; and there are marked gender differences in the rate at which tolerance develops to the daily administration of PK 11195 as well as gender differences in several PK-11195- and/or flumazenil-induced withdrawal signs in DZ-dependent rats. These data suggest that both pharma-

codynamic factors such as the hormonal milieu, changes in homeostatic relationships of neurostransmitter systems, and modulation of receptors and pharmacokinetic factors are probably involved in these observed effects although the exact mechanisms through which these changes occur have not been elucidated.

ACKNOWLEDGEMENTS

The authors wish to thank Hoffmann-LaRoche, Inc., Nutley, N.J. for their generosity in supplying diazepam and flumazenil; Karen Casey and Wayne Fielder for technical assistance in preparation of the manuscript.

REFERENCES

1. Awad, M.; Gavish, M.: Binding of [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 to cerebral cortex and peripheral tissues of various species: Species differences and hererogeneity in peripheral benzodiazepine binding sites. J. Neurochem. 49:1407–1414; 1987.
2. Benavides, J.; Capdeville, C.; Dauphin, F.; Dubois, A.; Duverger, D.; Fage, D.; Gotti, B.; MacKenzie, E. T.; Scatton, B.: The quantification of brain lesions with an φ_2 site ligand: a critical analysis of animal models of cerebral ischaemia and neurodegeneration. Brain Res. 522:275–289; 1990.
3. Benavides, J.; Quarteronet, D.; Imbault, F.; Malgouris, C.; Uzan, A.; Renault, C.; Dubroeucq, M.; Gueremy, C.; Le Fur, G.: Labeling of "peripheral-type" benzodiazepine binding sites in the rat brain by using [^{3}H] PK 11195, an isoquinline carboxamide derivative: Kinetic studies and autoradiographic localization. J. Neurochem. 41:1744–1750; 1983.
4. Bitran, D.; Carlson, D.; Leschiner, S.; Gavish, M.: Ovarian steroids and stress produce changes in peripheral benzodiazepine receptor density. Eur. J. Pharmacol. 361:235–242; 1998.
5. Bitran, D.; Dowd, J. A.: Ovarian steroids modify the behavioral and neurochemical responses of the central benzodiazepine receptor. Psychopharmacology (Berl) 125:65–73; 1996.
6. Butera, P. C.; Bradway, D. M.; Cataldo, N. J.: Modulation of satiety effect of cholecystokinin by estradiol. Physiol. Behav. 53:1235–1238; 1993.
7. Butera, P. C.; Xiong, M.; Davis, R. J.; Platania, S. P.: Central implants of dilute estradiol enhance the satiety effect of CCK-8. Behav. Neurosci. 110:823–830; 1996.
8. Byrnes, J. J.; Miller, L. G.; Perkins, K.; Greenblatt, D. J.; Shader, R. I.: Chronic benzodiazepine administration XI: Concurrent administration of PK 11195 attenuates lorazepam discontinuation effects. Neuropsychopharmacology 8:267–273; 1993.
9. Calvo, D. J.; Campos, M. B.; Calandra, R. S.; Medina, J. H.; Ritta, M. N.: Effect of long term diazepam administration on testicular benzodiazepine receptors and steroidogenesis. Life Sci. 49:519–523; 1991.
10. Chiu, T. H.; Rosenberg, H. C.: Barbiturates and benzodiazepines: Effects and mechanisms. In: Chang, L. W.; Dyer, R. S., eds. Handbook of neurotoxicology. New York: Marcel Dekker, Inc.; 1995:739–767.
11. Cooper, S. J.: Benzodiazepines as appetite-enhancing compounds. Appetite 1:7–19; 1980.
12. Diaz-Garcia, J. M.; Oliver-Botana, J.; Fos-Galve, D.: Pharmacokinetics of diazepam in the rat: influence of a carbon tetracholoride-induced hepatic injury. J. Pharm. Sci. 81:768–772; 1992.
13. Drugan, R. C.: Peripheral benzodiazepine receptors: Molecular pharmacology to possible physiological significance in stress-induced hypertension. Clin. Neuropharmacol. 19:475–496; 1996.
14. Fernandes, C.; File, S. E.; Berry, D.: Evidence against oppositional and pharmacokinetic mechanisms of tolerance to diazepam's sedative effects. Brain Res. 734:236–242; 1996.
15. Ferrarese, C.; Appollonio, I.; Bianchi, G.; Frigo, M.; Marzorati, C.; Pecora, N; Perego, M.; Pierpaoli, C.; Frattola, L.: Benzodiazepine receptors and diazepam binding inhibitor: A possible link between stress, anxiety and the immune system. Psychoneuroendocrinology 18:3–22; 1993.
16. File, S. E.: Pro- and anti-convulsant properties of PK 11195, a ligand for benzodiazepine binding sites: Development of tolerance. Br. J. Pharmacol. 83:471–476; 1984.
17. File, S. E.; Pellow, S.: The effects of PK 11195, a ligand for benzodiazepine binding sites, in animal tests of anxiety and stress. Pharmacol Biochem Behav. 23:737–741; 1985.
18. File, S. E.: The history of benzodiazepine dependence. A review of animal studies. Neurosci. Biobehav. Rev. 14:135–146; 1990.
19. Flanagan-Cato, L. M.; King, J. L.; Blechman, J. G.; O'Brien, M. P.: Estrogen reduces cholecystokinin-induced c-Fos expression in the rat brain. Neuroendocrinology 67:384–391; 1998.
20. Gallager, D. W.; Primus, R. J.: Benzodiazepine tolerance and dependence: $GABA_A$ receptor complex locus of change. In: Wonnacott, S.; Glund, G., eds. Neurochemistry of drug dependence. London: Portland Press; 1993:135–151.
21. Gallager, D. W.; Heninger, K.; Heninger, G.: Periodic benzodiazepine antagonist administration prevents benzodiazepine withdrawal symptoms in primates. Eur. J. Pharmacol. 132:31–38; 1986.
22. Gallager, D. W.; Malcolm, A. B.; Anderson, S. A.; Gonsalves, S. F.: Continuous release of diazepam: electrophysiological, biochemical and behavioral consequences. Brain Res. 342:26–36; 1985.
23. Gavish, M.; Bar-Ami, S.; Weizman, R.: Pathophysiological and endocrinological aspects of peripheral-type benzodiazepine receptors. In: Jenner, P., series ed. Neuroscience Perspectives, & Giesen-Crouse, E., vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press; 1993: 209–234.
24. Gavish, M.; Bar-Ami, S.; Weizman, R.: The endocrine system and mitochondrial benzodiazepine receptors. Mol Cell. Endocrinol. 88:1–13; 1992.
25. Gerak, L. R.; France, C. P.: Repeated administration of flumazenil does not alter its potency in modifying schedule-controlled behavior in chlordiazepoxide-treated rhesus monkeys. Psychopharmacol. 131:64–70; 1997.
26. Greenblatt, D. J.; Allen, M. D.; Harmatz, J. S.; Shader, R. I.: Diazepam disposition determinants. Clin Pharmacol. Ther. 301–312, 1980.
27. Greenblatt, D. J.; Divoll, M.; Harmatz, J. S.; Shader, R. I.: Oxazepam kinetics: Effects of age and sex. J. Pharmacol. Exp. Ther. 215:86–91; 1980.
28. Grimm, V. E.; Jancourt, A.: The effects of chronic diazepam treatment on body weight and food intake in rats. Intern. J. Neurosci. 18:127–36; 1983.
29. Hegarty, A. A.; Vogel, W. H.: The effect of acute and chronic diazepam treatment on stress-induced changes in cortical dopamine in the rat. Pharmacol. Biochem. Behav. 52:771–778; 1995.
30. Higgs, S.; Gilbert, D. B.; Barnes, N. M.; Cooper, S. J.: Possible brainstem mediation of benzodiazepine-induced hyperphagia. Appetite 21:183; 1993.
31. Honack, D.; Losher, W.: Sex differences in NMDA receptor mediated responses in rats. Brain Res. 620:167–170; 1993.
32. Itzhak, Y.; Norenberg, M. D.: Attenuation of ammonia toxicity in mice by PK 11195 and pregnenolone sulfate. Neurosci. Lett. 182:251–254; 1994.
33. Jing, X.; Wala, E. P.; Sloan, J. W.: The effect of chronic benzodiazepine exposure on body weight in rats. Pharmacological Res. 37:179–189; 1998.
34. Jing, X.; Wala, E. P.; Sloan, J. W.: The effect of PK 11195, an antagonist of the peripheral benzodiazepine receptors, on body weight in rats chronically exposed to diazepam. Pharmacological Res. (accepted for publication).
35. Joseph-Liauzun, E.; Farges, R.; Delamas, P.; Ferrara, P.; Loison, G.: The Mr 18,000 subunit of the peripheral-type benzodiazepine receptor exhibits both benzodiazepine and isoquinoline carboxamide binding sites in the absence of the voltage-dependent anion channel or of the adenine nucleotide carrier. J. Biol. Chem. 272:28102–28106; 1997.

36. Kepler, K. L.; Standifer, D.; Paul, D.; Kest, B.; Pasternak, G. W.; Bodner, R. J.: Gender effects and central opiod analgesia. Pain 45:87–94; 1991.
37. Kiyatkin, E. A.; Rebec, G. V.: Modulation of striatal neuronal activity by glutamate and GABA: iontophoresis in awake unrestrained rats. Brain Res. 822:88–106; 1999.
38. Koff, J. M.; Pritchard, G. A.; Greenblatt, D. J.; Miller, L. G.: The NMDA receptor competitive antagonist CPP modulates benzodiazepine tolerance and discontinuation. Pharmacology 55:217–227; 1997.
39. Krueger, K. E.: Peripheral-type benzodiazepine receptors: A second site of action for benzodiazepines. In: Costa, E., ed. Current concepts on receptor and enzyme regulation. American College of Neuropsychopharmacology. New York: Elsevier Science Publishing Co., Inc.; 1991:237–244.
40. Krueger, K. E.; Papadopoulos, V.: The role of mitochondrial benzodiazepine receptors in steroidogenesis. In: Jenner, P., series ed. Neuroscience perspectives, & Giesen-Crouse, E., vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press; 1993:89–109.
41. Lamb, R. J.; Griffiths, R. R.: Effects of repeated Ro 15-1788 administration on benzodiazepine-dependent baboons. Eur J. Pharmacol 110:257–261; 1985.
42. Langer, S. Z.; Arbilla, S.: Limitaions of the benzodiazepine receptor nomenclature: A proposal for a pharmacological classification as omega receptor subtypes. Fund. Clin. Pharmacol. 2:159–170; 1988.
43. Laviano, A.; Meguid, M. M.; Gleason, J. R.; Yang, Z.-J.; Renvyle, T.: Comparison of long-term feeding pattern between male and female Fisher 344 rats: influence of estrous cycle. Am. J. Physiol. 270:R413–419; 1996.
44. Lukas, S. E.; Griffiths, R. R.: Precipitated diazepam withdrawal in baboons: Effects of dose and duration of diazepam exposure. Eur. J. Pharamacol. 100:163–171; 1984.
45. Martin, J. R.; Haefely, W. E.: Drugs used for the treatment of anxiety. In: Munson, P. L.; Mueller, R. A.; Breese, G. R., eds. Principles of pharmacology: Basic concepts & clinical applications. New York: Chapman & Hall; 1995:243–247.
46. Martin, J. R.; Moreau, G.; Jenck F.: Precipitated withdrawal in squirrel monkeys after repeated daily oral administration of alprazolam, diazepam, flunitrazepam or oxazepam. Psychopharmacology 118:273–279; 1995.
47. Martin, W. R.; McNicholas, L. F.; Pruitt, T. A.: Physical dependence of benzodiazepines in the rat and dog. NIDA Res. Monogr. 67:202–208; 1986.
48. Martin, W. R.; Sloan, J. W.; Wala, E. P.: Precipitated abstinence in orally dosed benzodiazepine-dependent dogs. J. Pharmacol. Exp. Ther. 255:744–755; 1990.
49. Martin, W. R.; Sloan, J. W.; Wala, E. P.: Precipitated abstinence in the diazepam-dependent rat. Pharmacol. Biochem Behav. 46:683–688; 1993.
50. Martinez, J. A.; Fargeas, M. J.; Bueno, L.: Physical dependence on diazepam: Precipitation of abstinence syndromes by peripheral and central benzodiazepine receptor antagonists. Pharmacol. Biochem. Behav. 41:461–464; 1992.
51. Miller, L. G.; Koff, J. M.: Interaction of central and peripheral benzodiazepine sites in benzodiazepine tolerance and discontinuation. Prog. Neuropsychopharmacol. Biol. Psychiatry 18:847–857; 1994.
52. Mizoule, J.; Gauthier, A.; Uzan, A.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Le Fur, G.: Opposite effects of two ligands for peripheral type benzodiazepine binding sites, PK 11195 and RO5-4864, in a conflict situation in the rat. Life Sci. 36:1059–1068; 1985.
53. Morrow, A. L.; Pace, J. R.; Purdy, R. H.; Paul, S. M.: Characterization of steroid interactions with GABA receptor-gated chloride ion channels. Mol. Pharmacol. 37:262–270; 1990.
54. Myers, R.: Mitochondrial benzodiazepine receptor ligands as indicators of damage in the CNS: Their application in positron emission tomography. In: Jenner, P., series ed. Neuroscience perspectives, & Giesen-Crouse, E., vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press, 1993:235–273.
55. Nakamoto, Y.; Watabe, S.; Shiotani, T.; Yoshii, M.: Peripheral-type benzodiazepine receptors in association with epileptic seizures in ELmice. Brain Res. 717:91–98; 1996.
56. Parola, A. L.; Yamamura, H. I.: Molecular properties of mitochondrial benzodiazepine receptors. In: Jenner, P., series ed. Neuroscience perspectives, & Giesen-Crouse, E.,vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press; 1993:3–26.
57. Pawlikowski, M.: Immunomodulating effects of peripherally acting benzodiazepines. In: Jenner, P., series ed. Neuroscience perspectives, & Giesen-Crouse, E., vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press; 1993:125–135.
58. Pecina, S.; Berridge, K. C.: Brainstem mediates diazepam enhancement of palatability and feeding: microinjections into fourth ventricle versus lateral ventricle. Brain Res. 727:22–30; 1996.
59. Pesce, M. E.; Acevedo, X.; Pinardi, G.; Miranda, H. F.: Gender differences in diazepam withdrawal syndrome in mice. Pharmacol. Toxicol. 75:353–355; 1994.
60. Pritchett, D. B.; Seeburg, P. H.: (-Aminobutyric $Acid_A$ receptor α5-subunit creates novel type II benzodiazepine receptor pharmacology. J. Neurochem 54:1802–1804; 1990.
61. Pritchett, D. B.; Sontheimer, H.; Shivers, B. D.; Ymer, S.; Kettenmann, H.; Schofield, P. R.; Seeburg, P. H.: Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338:580–585; 1989.
62. Puia, G.; Santi, M.; Vincini, S.; Pritchett, D. B.; Purdy, R. H.; Paul, S. M.; Seeburg, P. H.; Costa, E.: Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 4:759–765; 1990.
63. Ramsey-Williams, V. A.; Wu, Y.; Rosenberg, H. C.: Comparison of anticonvulsant tolerance, crosstolerance, and benzodiazepine receptor binding following chronic treatment with diazepam or midazolam. Pharmacol. Biochem. Behav. 48:765–772; 1994.
64. Reddy, D. S.; Kulkarni, S. K.: The role of GABA-A and mitochondrial diazepam-binding inhibitor receptors on the effects of neurosteroids on food intake in mice. Psychopharmacology 137:391–400; 1998.
65. Reilly, P. E.; Thompson, D. A.; Mason, S. R.; Hooper, W. D.: Cytochrome P450IIIA enzymes in rat liver microsomes: Involvement in C3-hydroxylation of diazepam and nordazepam but not N-dealkylation of diazepam and temazepam. Mol. Pharmacol. 37:767–774; 1990.
66. Saano, V.: Effects of peripherally acting benzodiazepines on cell growth and differentiation. In: Jenner, P., series ed. Neuroscience perspectives, & Giesen-Crouse, E., vol. ed. Peripheral benzodiazepine receptors. New York: Academic Press; 1993:111–124.
67. Sillence, M. N.; Reich, M. M.; Thomson, B. C.: Sexual dimorphism in the growth response of entire and gonadectomized rats to clenbuterol. Am. J. Physiol. 268:E1077–1082; 1995.
68. Sloan, J. W.; Martin, W. R.; Wala, E. P.: A comparison of the physical dependence inducing properties of flunitrazepam and diazepam. Pharmacol. Biochem. Behav. 39:395–405; 1991.
69. Sloan, J. W.; Wala, E. P.: Pharmacology of sedatives, hypnotics, and anxiolytics. In: Tarter, R. E.; Ammerman, R. T.; Ott, P. J., eds. Handbook of substance abuse: Neurobehavioral pharmacology. New York: Plenum Press; 1998:395–433.
70. Sloan, J. W.; Wala, E.; Jing, X.; Holtman, J. R. Jr.; Milliken, B.: Diazepam-treated female rats: Flumazenil-and PK 11195-induced withdrawal in the Hippocampus CA1. Pharmacol. Biochem. Behav. 61:121–130; 1998.
71. Stock, H.; Ford, K.; Biscardi, R.; Wilson, M. A.: Lack of sex differences in anxiety behaviors during precipitated benzodiazepine withdrawal in rats. Physiol. Behav. 66:125–130; 1999.
72. Strobel, B. A.: Anatomical distribution of NADPH-cytochrome P450 reductase and cytochrome P4502D forms in rat brain: effects of xenobiotics and sex steroids. Mol. Cell. Biochem. 162:31–41; 1996.
73. Tsang, C.-F. C.; Wilkinson, G. R.: Diazepam disposition in mature and aged rabbits and rats. Drug Metab. Disp. 10:413–416; 1982.
74. Tsuda, M.; Chiba, Y.; Tsutomu, S.; Misawa, M.: Upregulation of NMDA receptor subunit proteins in the cerebral cortex during diazepam withdrawal. Eur. J. Pharmacol. 341:R1–R2; 1998.
75. Tsuda, M.; Suzuki, T.; Misawa, M.: NMDA receptor antagonists

potently suppress the spontaneous withdrawal signs induced by discontinuation of long-term diazepam treatment in Fischer 344 rats. Brain Res. 790:82–90; 1998.
76. Upton, N.; Blackburn, T.: Pharmacology of mammalian $GABA_A$ receptors. In: Enna, S. J.; Bowery, G., eds. The GABA receptors. 2nd ed. Totowa, NJ: Humana Press; 1997: 83–120.
77. Vallerand, A. H.: Gender differences in pain. Image J. Nurs. Sch. 27:235–237; 1995.
78. Wala, E. P.; Sloan, J. W.; Jing, X.: Comparison of abstinence syndromes precipitated by flumazenil and PK 11195 in female diazepam-dependent rats. Psychopharmacology 133:214–223; 1997.
79. Wala, E. P.; Sloan, J. W.; Jing, X.: Dorsal raphe and substantia nigra response to flumazenil in diazepam-dependent rats. Pharmacol. Biochem. Behav. 58:221–229; 1997.
80. Wala, E. P.; Sloan, J. W.; Jing, X.: Kinetics of behavioral and EEG manifestations of withdrawal syndromes induced by flumazenil and PK 11195 microinjected into the brain in rats physically dependent on diazepam. Scientific Meeting Polish Soc. Pharm. Abstracts, 17:229–230; 1998.
81. Wala, E. P.; Sloan, J. W.; Jing, X.; Holtman, P. H.: Intrathecally administered flumazenil and PK 11195 precipitate abstinence syndrome in freely moving diazepam dependent rats. Drug Alcohol Depend. 43:169–177; 1996.
82. Wala, E. P.; Sloan, J. W.; Jing, X.: Pharmacokinetics of PK 11195, an antagonist of the peripheral benzodiazepine receptors, in rats. The effect of dose and gender. Pharmacological Res. (In Press).
83. Watanabe, M.; Tanaka, M.; Tateishi, T.; Nakura, H.; Kumai, T.; Kobayashi, S.: Effects of the estrous cycle and the gender difference on hepatic drug-metabolising enzyme activities. Pharmacol. Res. 35:477–480; 1997.
84. Weizman, R.; Gavish, M.: Chronic diazepam treatment induces an increase in peripheral benzodiazepine binding sites. Clin. Neuropharmacol. 12:346–351; 1989.
85. Weizman, R.; Leschiner, S.; Schlegel, W.; Gavish, M.: Peripheral-type benzodiazepine receptor ligands and serum steroid hormones. Brain Res. 772:203–208; 1997.
86. Whitehouse, B. J.: Benzodiazepines and steroidogenesis. J. Endocrinol. 134:1–3; 1992.
87. Wilson, M. A.: Influences of the hormonal milieu on acute and chronic benzodiazepine responses in rats. In: Watson, R.R., ed. Drug of abuse and neurobiology. Ann Arbor: CRC Press; 1992:209–231.
88. Wilson, M. A.; Biscardi, R.: Effects of gender and gonadectomy on responses to chronic benzodiazepine receptor agonist exposure in rats. Eur. J. Pharmacol. 215:99–107; 1992.
89. Wilson, M. A.; Biscardi, R.: Influence of gender and brain region on neurosteroid modulation of GABA responses in rats. Life Sci. 60(19):1679–1691; 1997.
90. Wilson, M. A.; Gallager, D. W.: Ro 15-1788-induced seizures in rats continually exposed to diazepam for prolonged periods. Epilepsy Res. 2:14–19; 1988.
91. Wilson, M. A.; Gallager, D. W.: Effects of chronic diazepam exposure on GABA sensitivity on benzodiazepine potentiation of GABA-mediated responses of substantia nigra pars reticulata neurons of rats. Eur. J. Pharmacol. 136:333–343; 1987.
92. Wiviott, S.; Wiviott-Tishler, L.; Hyman, S. E.: Sedative-hypnotics and anxiolytics. In: Friedman, L.; Fleming, N. F.; Roberts, D. H.; Hyman, S. E., eds. Source book of substance abuse and addiction. Baltimore, MD: Williams & Wilkins; 1996:203–215.
93. Woods, J. H.; Katz, J. L.; Winger, G.: Benzodiazepines: Use, abuse and consequences. Pharmacol. Rev. 44:151–347; 1992.