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Ovarian steroids and stress produce changes in peripheral benzodiazepine receptor density

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Abstract

Although past research has described changes in the density of the peripheral benzodiazepine receptor in brain and in peripheral organs in response to stressors and steroid hormone exposure, their combined influence had yet to be determined. This study examined the effect of swim-stress as a function of ovarian hormone administration on the binding of an isoquinoline carboxamide derivative, [^{3}H]PK 11195, in brain and peripheral tissues. In olfactory bulb and adrenal gland, stress increased peripheral benzodiazepine receptor density in ovariectomized rats with and without estradiol and progesterone replacement injection, even when compared with unstressed animals treated with hormones, where estradiol + progesterone decreased peripheral benzodiazepine receptor number in olfactory bulb, but estradiol and estradiol + progesterone increased it in adrenal gland. In frontal cortex, stress decreased peripheral benzodiazepine receptor number, an effect that was reversed by estradiol. In hippocampus estradiol decreased peripheral benzodiazepine receptor density in unstressed animals and estradiol + progesterone decreased peripheral benzodiazepine receptor number in unstressed and stressed animals. In cerebellum, stress, estradiol and estradiol + progesterone alone decreased peripheral benzodiazepine receptor density. In uterus of unstressed controls, estradiol + progesterone increased peripheral benzodiazepine receptor density, and stress produced a further increase in steroid-treated females. Stress did not affect peripheral benzodiazepine receptor density in kidney, except in animals that received estradiol + progesterone injections, where swim-stress produced a significant decrease in peripheral benzodiazepine receptor density. Thus, steroid hormones regulate peripheral benzodiazepine receptor density in endocrine organs and brain, and the hormonal state of the organism modifies the peripheral benzodiazepine receptor response to stress in a tissue- and brain region-specific manner, suggesting that the peripheral benzodiazepine receptor may play a pivotal role in an integrated response to stress.

Keywords: Neurosteroid; Mitochondrial steroidogenesis; [^{3}H]PK 11195; Estrogen; Progesterone

1. Introduction

Since before the discovery of benzodiazepine receptor in the 1970s (Braestrup and Squires, 1977; Möhler and Okada, 1977), benzodiazepines have been extensively investigated for their wide ranging effects on the brain and spinal cord. The anxiolytic, ataxic, sedative, and anticonvulsant effects are mediated by the 'central benzodiazepine receptor'—a binding site that allosterically modulates C1-channel gating by $GABA_A$ receptors in the central nervous system. Evidence from a number of studies has raised the possibility that certain benzodiazepines may also influence brain function indirectly by binding to the 'peripheral benzodiazepine receptor'—a binding site found on the mitochondria of glial cells in the brain (Anholt et al., 1986; Mukhin et al., 1989; McEnery, 1992). The peripheral benzodiazepine receptor was originally described in peripheral tissues, such as the kidney, heart, adrenal cortex, testes, and ovaries, hence the term 'peripheral', to distinguish it from the neuronal central benzodiazepine receptor (Papadopoulos et al., 1991). In contrast to the central benzodiazepine receptor, the brain peripheral benzodiazepine receptor is not coupled to the $GABA_A$ receptor, and shows a different pharmacological profile from the central benzodiazepine receptor. Although central benzodiazepine receptor and peripheral benzodiazepine receptor bind diazepam with high affinity, clonazepam and flumazenil have a high affinity for the central benzodiazepine receptor, and display a low affinity for the periph-

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eral benzodiazepine receptor. Other ligands, such as RO5-4864 (4′-chlorodiazepam) and PK 11195 (an isoquinoline carboxamide derivative), show a high affinity for the peripheral benzodiazepine receptor and a low affinity for the central benzodiazepine receptor.

A well-studied effect of peripheral benzodiazepine receptor activation is the stimulation of steroid production via the resulting translocation of cholesterol from the outer to the inner mitochondrial membrane (Papadopoulos, 1993). A steroidogenic role for the peripheral benzodiazepine receptor in the brain has also been described (Korneyev et al., 1993; Romeo et al., 1993), thus leading to the production of neurosteroids—steroid hormones produced in brain, and often affecting brain function rapidly by interacting with membrane-bound neurotransmitter receptors (Romeo et al., 1993). One well-studied neurosteroid is allopregnanolone, a reduced metabolite of progesterone that has been shown to exert anxiolytic, anticonvulsant, ataxic, and hypnotic effects on behavior thus mimicking the actions of the pharmacotherapeutic benzodiazepines [reviewed by Bitran et al., 1991]. The mechanism underlying allopregnanolone's behavioral effects appears to be result from a potentiation of neuronal inhibition via brain $GABA_A$ receptors (Bitran et al., 1995).

Little is known about the functional significance of the peripheral benzodiazepine receptor in the brain for behavioral endpoints that are affected by $GABA_A$ receptor-mediated changes in neural excitability. A variety of stressors has been demonstrated to alter peripheral benzodiazepine receptor density and neurosteroid synthesis. In the central nervous system (CNS) and peripheral tissues of rats and mice, changes in peripheral benzodiazepine receptor density occur in response to inescapable shock (Drugan et al., 1986, 1988), forced swimming (Novas et al., 1987; Rago et al., 1989; Burgin et al., 1996), maximal electroshock (Basile et al., 1987), noise stress (Mennini et al., 1989; Ferrarese et al., 1991), chronic and constant exposure to light (Weissman et al., 1984), conditioned fear (Holmes et al., 1992), and injection of an anxiogenic β-carboline (Drugan et al., 1995). In humans, stress from examination or war, as well as electroconvulsive shock therapy were found to modify peripheral benzodiazepine receptor binding in blood platelets (Karp et al., 1989; Weizman et al., 1994, 1996).

A functional role of the peripheral benzodiazepine receptor in mediating stress-related responses is suggested by the finding that stressors increase the level of allopregnanolone in blood and brain (Purdy et al., 1991; Barbaccia et al., 1996a,b,c, 1997). Thus, stressors are seen as activators of neurosteroid synthesis, providing the $GABA_A$ receptor with a positive modulatory effect, thereby facilitating coping responses (i.e., anxiolytic, tranquilizing, anticonvulsant effects) to the stressful situation.

The overwhelming majority of the literature addressing the effect of stressors on peripheral benzodiazepine receptor density has been conducted on male rats. The studies documenting the modulatory role of ovarian steroids on peripheral benzodiazepine receptor binding have focused on endocrine organs. Thus, a gap in knowledge exists about the putative effects of ovarian steroids on brain peripheral benzodiazepine receptor populations. In addition, the potential interaction between ovarian hormones and stressors remains to be determined. Thus, in the following experiment, ovariectomized rats were given ovarian hormone injections that mimic physiological levels of the steroid, and peripheral benzodiazepine receptor density was examined in several peripheral tissues and dissected brain regions in animals that were exposed to swim stress.

2. Materials and methods

Adult Wistar female rats weighing 200–250 g at the beginning of the experiment were housed in temperature-controlled quarters with a 12-h light/dark cycle (lights on at 0700 h), with rat chow and water available ad libitum. Ovariectomy was conducted under ketamine HCl (60 mg/kg, i.p.) and xylazine HCl (10 mg/kg, i.p.) anesthesia. One week later, 36 females were randomly assigned to one of three steroid treatment groups: estradiol (20 μg/0.1 ml, s.c., 48 h prior to sacrifice), estradiol (48 h prior to sacrifice) plus progesterone (0.5 mg/0.1 ml, s.c., 4 h prior to sacrifice), or sesame seed oil vehicle (0.1 ml, s.c., at 48 and 4 h prior to sacrifice). Each group was further subdivided into one of two subgroups: swim stress, animals were placed in a pool of ambient temperature water for 5 min, or unstressed controls. All animals were handled daily after ovariectomy by being positioned in the guillotine for a 5-s period. Immediately after the swim stress, animals were killed by decapitation. Brains were rapidly removed and olfactory lobe, frontal cortex, hippocampus, and cerebellum were dissected on ice. Uteri, adrenal glands and kidneys were also removed. All tissues were rapidly frozen in liquid nitrogen and stored at −70°C until assay. Tissues were homogenized in 20–30 vols. of 50 mM ice-cold Tris–HCl buffer, pH 7.4, using a Brinkman Polytron (setting 10) for 15 s. The homogenate was centrifuged at 30,000 × *g* for 30 min. The pellet was re-suspended and re-centrifuged as above. The final pellets were suspended in Tris–HCl buffer to reach the following protein concentrations: 500 μg/ml in olfactory bulb, 1.25 mg/ml in frontal cortex, 800 μg/ml in hippocampus, 1 mg/ml in cerebellum, 110 μg/ml in uterus, 62 μg/ml in adrenal gland, and 440 μg/ml in kidney. A 400 μl aliquot of membranes was incubated with 25 μl of [^{3}H]PK 11195 (Spec. Act. 83.5 Ci/mmol, New England Nuclear, Boston, MA) in each of six concentrations (0.187–6 nM) in the absence (total binding) or presence (non-specific binding) of 75 μl unlabelled PK 11195 (10 μM final concentration) for 1 h in a 4°C ice-water bath. Duplicate samples were filtered under vacuum over Whatman GF/B filters using a

Table 1
Equilibrium dissociation constant (K_d) for [^{3}H]PK 11195 at the peripheral benzodiazepine receptor in olfactory bulb, frontal cortex, hippocampus, cerebellum, adrenal gland, uterus, and kidney of ovariectomized rats injected with estradiol, estradiol and progesterone, or sesame oil vehicle as a function of swim stress

TRT	Condition	Structure						
		Olf bulb	Frtl cx	Hippoc	Cbl	Adrenal	Uterus	Kidney
Oil	Control	2.59 ± 0.19	1.92 ± 0.15	1.82 ± 0.10	2.31 ± 0.16	1.81 ± 0.05	0.97 ± 0.09	1.69 ± 0.06
	Stress	2.42 ± 0.25	1.78 ± 0.14	1.62 ± 0.08	2.77 ± 0.10	1.73 ± 0.22	0.77 ± 0.04	1.57 ± 0.09
E_2	Control	2.33 ± 0.24	1.52 ± 0.06	1.72 ± 0.04	2.25 ± 0.16	1.91 ± 0.11	0.72 ± 0.09	1.60 ± 0.33
	Stress	2.08 ± 0.10	1.61 ± 0.05	1.80 ± 0.13	2.65 ± 0.25	1.45 ± 0.14	0.79 ± 0.07	1.63 ± 0.24
E_2 + P	Control	2.68 ± 0.13	1.72 ± 0.15	1.63 ± 0.04	2.24 ± 0.11	1.64 ± 0.12	0.85 ± 0.09	1.44 ± 0.13
	Stress	2.57 ± 0.19	1.96 ± 0.18	1.48 ± 0.13	2.65 ± 0.27	1.96 ± 0.19	1.04 ± 0.13	1.29 ± 0.34

K_d (nM) expressed as the mean ± S.E.M. derived from saturation experiments performed on tissues obtained from six animals per treatment ($N = 36$ total).

Brandel M-18R filtering manifold and washed three times with 3–5 ml of 50 mM ice-cold Tris–HCl buffer. Radioactivity was counted using a Beckman LS 6500 liquid scintillation spectrometer using 5 ml of Eco-scint solution. Protein concentration was determined as previously described (Markwell et al., 1981). For each of the seven target structures, peripheral benzodiazepine receptor density (B_{max}) and equilibrium dissociation constant (K_d) data were analyzed using separate three by two analysis of variance (ANOVA). Main effects were further tested using Bonferroni adjusted pairwise comparisons. Significant interaction terms were probed using post-hoc simple effect contrasts. Statistical significance was attributed with a $P < 0.05$.

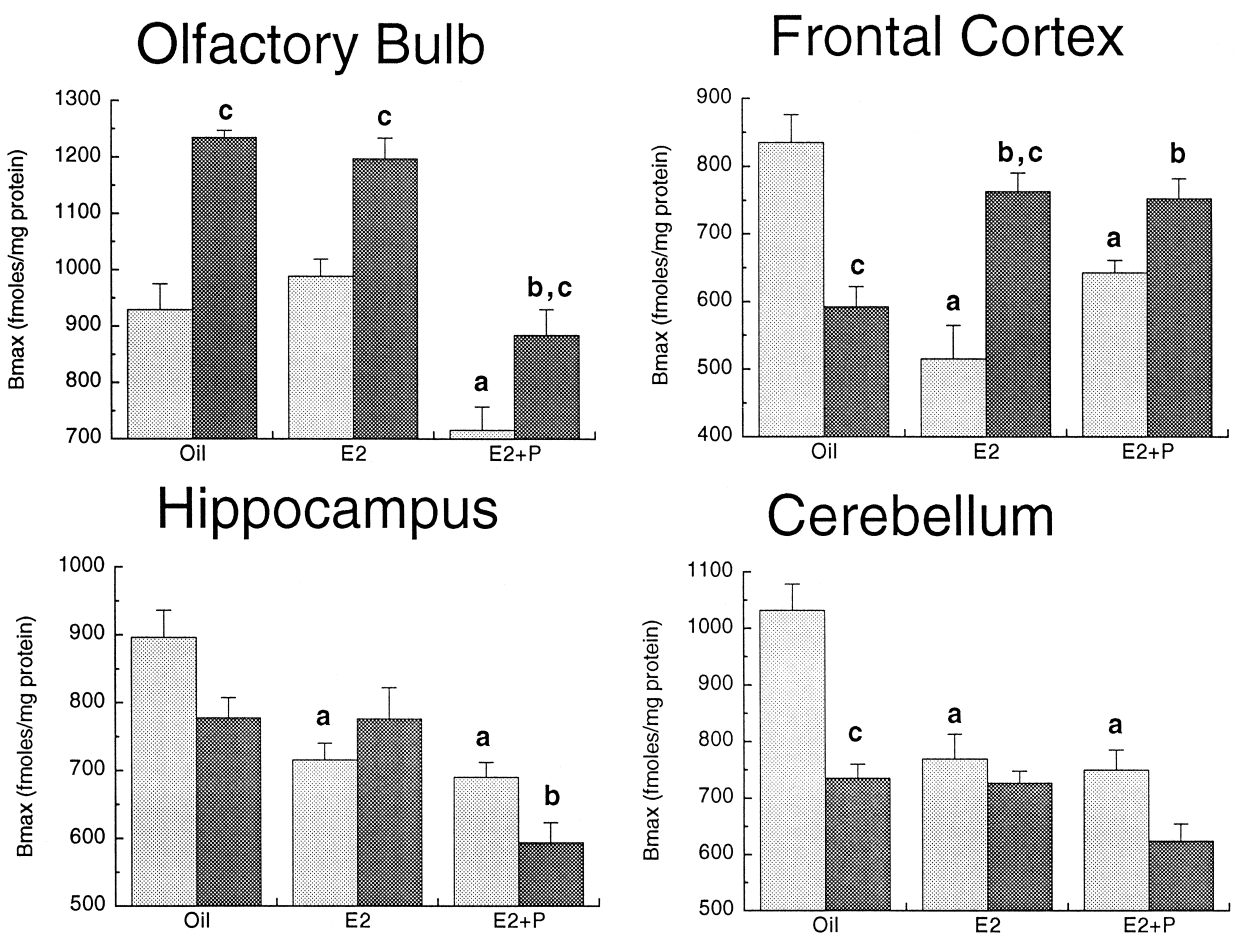

Fig. 1. Maximal binding density (B_{max}) for the peripheral benzodiazepine receptor using [^{3}H]PK 11195 in homogenates of selected brain regions from control (▨) or stressed (▩) ovariectomized female rats treated with oil, estradiol, or estradiol and progesterone. See text for further details about stressor parameters and injection regiment. Significant differences indicated are as follows: (a) relative to control oil; (b) relative to stress oil; (c) relative to respective control group.

3. Results

ANOVA revealed that the K_d for [^{3}H]PK 11195 binding to the peripheral benzodiazepine receptor was not affected by steroid treatment or exposure to swim stress in any of the structures examined (Table 1). The effects of hormone injection and swim stress on peripheral benzodiazepine receptor density in the olfactory bulb, frontal cortex, hippocampus, and cerebellum are shown in Fig. 1. In the olfactory bulb of control and stress females, estradiol + progesterone treatment produced a 25–30% decrease in B_{max}, compared to oil-treated rats. In addition, swim stress increased peripheral benzodiazepine receptor density by 20–30% in oil-, estradiol- and estradiol + progesterone-treated groups. Statistical analysis confirmed these observations; a main effect of steroid treatment was found, $F(2,30) = 32.1$, $P < 0.001$, with a significant decrease in B_{max} in estradiol + progesterone treated animals relative to oil ($P < 0.001$) and estradiol-injected females ($P < 0.01$). A main effect of stress was also found, $F(1,30) = 50.8$, $P < 0.001$, but the steroid by stress interaction was not significant, $F(2,30) = 0.8$. In frontal cortex, hippocampus, and cerebellum, the effects of swim stress on peripheral benzodiazepine receptor density varied as a function of hormone treatment. A significant interaction of steroid injection and stress was revealed by ANOVA in frontal cortex, $F(2,30) = 27.7$, $P < 0.0001$; hippocampus, $F(2,30) = 4.38$, $P < 0.05$; and cerebellum, $F(2,30) = 6.76$, $P < 0.005$. Swim stress produced a 30% reduction in peripheral benzodiazepine receptor density in the frontal cortex and cerebellum of ovariectomized oil-treated animals, but did not affect significantly peripheral benzodiazepine receptor density in the hippocampus. In unstressed females, estradiol or estradiol + progesterone treatment decreased peripheral benzodiazepine receptor density in frontal cortex, hippocampus, and cerebellum by 20–40%. In contrast, brain region-specific effects of ovarian hormones on peripheral benzodiazepine receptor density were observed in animals exposed to swim stress. In frontal cortex of swim stressed females, estradiol or estradiol + progesterone injection increased the B_{max} by 28%, relative to stressed vehicle-treated females. In the hippocampus of stressed rats, estradiol + progesterone treatment decreased hippocampal peripheral benzodiazepine receptor density by 25%, relative to oil- or estradiol-treated animals. In the cerebellum of stressed animals, steroid treatment did not affect peripheral benzodiazepine receptor density.

The effects of ovarian hormone injection and swim stress on peripheral benzodiazepine receptor density in the adrenal gland, uterus, and kidney are shown in Fig. 2. In the adrenal gland of control and stressed females, an increase in B_{max} was observed after estradiol (~40%), and a further increase occurred after estradiol + progesterone treatment (~125%). Swim-stress yielded a 35–60% increase in B_{max} in each of the vehicle and steroid conditions. Statistical analysis of these data revealed a main effect of steroid treatment, $F(2,30) = 257.1$, $P < 0.0001$, a main effect of stress, $F(1,30) = 176.0$, $P < 0.0001$, and a significant steroid by stress interaction, $F(2,30) = 3.22$, $P < 0.05$. In the uterus and kidney, the effect of swim stress on peripheral benzodiazepine recep-

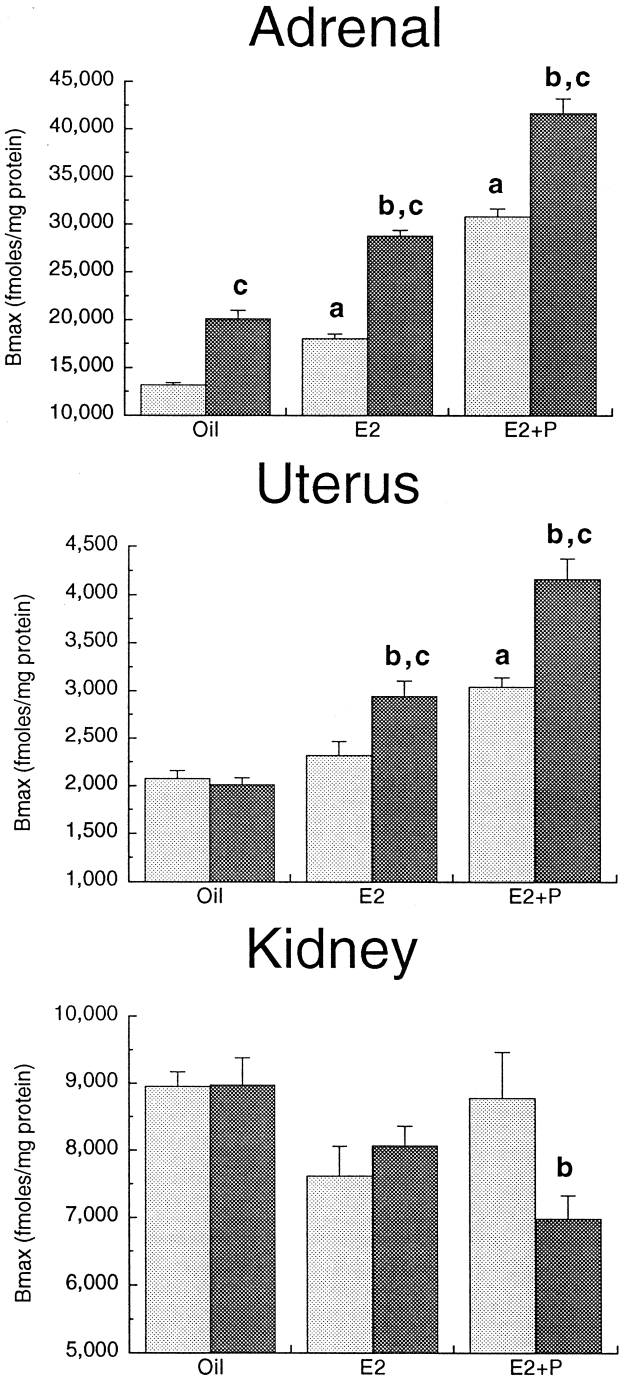

Fig. 2. Maximal binding density (B_{max}) for the peripheral benzodiazepine receptor using [^{3}H]PK 11195 in homogenates of peripheral tissues from control (▨) or stressed (▩) ovariectomized female rats treated with oil, estradiol, or estradiol and progesterone. See text for further details about stressor parameters and injection regiment. Significant differences indicated are as follows: (a) relative to control oil; (b) relative to stress oil; (c) relative to respective control group.

tor density was dependent on the hormone treatment. A significant interaction of steroid injection and stress was revealed by ANOVA in the uterus, $F(2,30) = 9.25$, $P < 0.001$, and in the kidney, $F(2,30) = 3.95$, $P < 0.05$. In unstressed rats, estradiol treatment did not significantly affect peripheral benzodiazepine receptor density in the uterus or kidney; however, estradiol + progesterone injection increased B_{max} for the peripheral benzodiazepine receptor by 46% in uterus, but had no effect in the kidney. Swim-stress did not affect peripheral benzodiazepine receptor density in the uterus or kidney of oil-treated control females. In the uterus of swim-stressed rats, a 50% increase in peripheral benzodiazepine receptor density resulted from estradiol treatment, and a 100% increase after estradiol + progesterone treatment, compared to oil-treated rats. In the kidney of swim-stressed females, estradiol had no effect on peripheral benzodiazepine receptor density, while estradiol + progesterone treatment produced a 33% decrease in the B_{max}, compared to oil-treated rats.

4. Discussion

The results clearly show that the effect of swim stress on peripheral benzodiazepine receptor density is dependent on the hormonal status of the organism, that the administration of physiological doses of ovarian steroids affects peripheral benzodiazepine receptor number, and that these effects are not uniformly found, but that a specificity exists for the tissue or brain region examined.

Peripheral benzodiazepine receptors in endocrine tissues have been shown to be responsive to fluctuations in the circulating level of gonadotropins (Fares et al., 1987), estradiol (Fares et al., 1987; Bar-Ami et al., 1989), and adrenocorticotropic hormone (Fares et al., 1989). In the present study, an increase in uterine peripheral benzodiazepine receptor number was found after estradiol treatment of stressed animals, and after estradiol + progesterone treatment of unstressed and stressed animals. However, ovarian hormone administration also increased peripheral benzodiazepine receptor density in the adrenal gland. Thus, an autocrine or paracrine regulation of peripheral benzodiazepine receptor in the adrenal gland by ovarian steroids is suggested, as has been previously reported following the administration of progesterone in the ovary (Bar-Ami et al., 1994). Changes in peripheral benzodiazepine receptor number in endocrine organs by ovarian steroids may represent a positive feedback mechanism regulating steroidogenic activity (Bar-Ami et al., 1994).

Our finding that steroid treatment alone did not affect peripheral benzodiazepine receptor density in the kidney is in contrast to the increase found after 10 days of treatment with a high dose of estradiol benzoate (2 mg/kg), or a combined treatment of estradiol benzoate + progesterone (Gavish et al., 1986, 1987). However, the use of male rats, high dose and/or the chronic nature of the steroid treatment may account for these differences. This suggestion is supported by the finding that renal peripheral benzodiazepine receptor binding remained unchanged across the estrous cycle or after treatment of female rats with a single dose of estradiol benzoate (Fares et al., 1987, 1988).

In all brain regions we examined, estradiol or estradiol + progesterone treatment decreased the number of peripheral benzodiazepine receptors. This result becomes all the more remarkable when contrasted against the inductive effect that these steroids had on peripheral benzodiazepine receptor number in steroid-sensitive peripheral organs. However, whereas ovarian steroids produced divergent effects on peripheral benzodiazepine receptor in brain and endocrine tissue, swim stress did not. Only in cerebellum was the hormonal-induced decrease also observed in stressed animals. Thus, in olfactory bulb and frontal cortex, stress attenuated or prevented the steroid-induced decrease in peripheral benzodiazepine receptor density, whereas in uterus and adrenal gland stress potentiated the steroid-induced increase.

The increase in peripheral benzodiazepine receptor number in olfactory bulb and adrenal gland that resulted from swim-stress in ovariectomized oil-treated animals is in good agreement with other reports of similar increases in these tissues (Novas et al., 1987; Ferrarese et al., 1991; Holmes et al., 1992; Drugan et al., 1995). The stress-induced induction of peripheral benzodiazepine receptor in adrenal and olfactory lobe has been hypothesized to contribute to an alarming reaction to the stressor (Drugan, 1996), increasing the substrate that regulates steroidogenesis (Krueger and Papadopoulos, 1992; Papadopoulos, 1993). As a result, the production of neurosteroids is facilitated (Purdy et al., 1991; Concas et al., 1996; ; Barbaccia et al., 1996a,b,c, 1997), thereby increasing GABAergic tone and promoting anxiolytic and anticonvulsant effects (Bitran et al., 1991, 1993, 1995; Wieland et al., 1991; Zimmerberg et al., 1994; Fernandez-Guasti and Picazo, 1995; Wieland et al., 1995).

The decreased number of peripheral benzodiazepine receptor found in frontal cortex in ovariectomized oil-treated females was unexpected since previous work had shown that mild stressors, comparable to that employed in this experiment, either increased (Mennini et al., 1989; Rago et al., 1989; Ferrarese et al., 1991) or failed to alter cortical peripheral benzodiazepine receptor binding capacity (Drugan et al., 1988, 1995; Weizman et al., 1990). A possible explanation for the discrepant results lies in the gonadal status of the organism at the time of the stressor. Thus, ovariectomized females administered estradiol or a combined estradiol + progesterone treatment showed the expected stress-induced decrease in cortical peripheral benzodiazepine receptor number previously reported in intact male rats.

To our knowledge, no other report exists on changes in peripheral benzodiazepine receptor density in the cerebellum as a result of stress. Excitatory effects of peripheral benzodiazepine receptor ligands on Purkinje neuron firing

rates have been reported (Basile et al., 1989), thus implicating a functional role of the peripheral benzodiazepine receptor in cerebellar physiology. In addition, the neurosteroid allopregnanolone, a product of brain peripheral benzodiazepine receptor stimulation (Korneyev et al., 1993; Romeo et al., 1993), has potent inhibitory effects on cerebellar Purkinje neurons (Smith et al., 1987; Smith, 1989). Thus, our findings predict that stressors would alter the activity pattern of cerebellar neurons as a result of changes in peripheral benzodiazepine receptor density.

Swim stress of ovariectomized oil-treated females was found to have no effect on peripheral benzodiazepine receptor density in hippocampus, uterus, or kidney. The lack of effect in hippocampus is in contrast to an earlier finding that noise stress increased hippocampal peripheral benzodiazepine receptor number (Ferrarese et al., 1991). Treatment of ovariectomized females with estradiol or estradiol + progesterone did not reveal an effect of the stressor. Thus, our results may represent a sexually dimorphic response where the hippocampus in females is not affected by stressors.

To date, the most reliable effect of stress on peripheral benzodiazepine receptor density has been found in the kidney, where the effect appears to vary with stressor intensity (Drugan et al., 1986, 1988; Novas et al., 1987; Armando et al., 1988; Rago et al., 1989; Weizman et al., 1990; Holmes et al., 1992). Yet in our experiments, stress of ovariectomized rats treated with oil or estradiol did not affect renal peripheral benzodiazepine receptor density. Only in estradiol + progesterone-treated animals was there a stress effect—and this consisted of a significant *decrease* in renal peripheral benzodiazepine receptor binding sites. Interestingly, a sexually dimorphic response of renal peripheral benzodiazepine receptor to stressors has been observed (Drugan et al., 1993). Whereas inescapable shock in the male rat decreased renal peripheral benzodiazepine receptor number, a diurnal variation in this response was found in female rats. Shock in the early part of the light cycle did not change renal peripheral benzodiazepine receptor density, but as the light cycle progressed a small but reliable decrease was reported. The lack of effect of swim stress on renal peripheral benzodiazepine receptor number in our experiment possibly is due to the fact that our animals were tested in the early part of the light cycle.

The mechanism(s) by which steroid hormones or stress produce changes in peripheral benzodiazepine receptor density remain unknown. The hormone replacement regimen that produced an upregulation of peripheral benzodiazepine receptor number includes a sufficient amount of time for the synthesis of additional peripheral benzodiazepine receptor sites and subsequent incorporation into mitochondria. Another possibility is that steroids may interact with the peripheral benzodiazepine receptor and modulate the binding of [^{3}H]PK 11195. To our knowledge, the in vitro effects of estradiol and progesterone on [^{3}H]PK 11195 binding has not been carefully ascertained. The changes in peripheral benzodiazepine receptor density observed after swim stress probably do not reflect changes in actual peripheral benzodiazepine receptor number since the animals were sacrificed immediately after swim stress. Thus, stress-induced changes in peripheral benzodiazepine receptor density may reflect more subtle alterations, such as change in peripheral benzodiazepine receptor turnover, masking or unmasking of binding sites, and receptor internalization or insertion.

Regardless of the actual mechanism(s) underlying the changes in [^{3}H]PK 11195 binding, when cast in light of other research concerned with the physiological consequences of peripheral benzodiazepine receptor stimulation, our results give rise to the following conclusions. Given its putative influence in steroidogenesis, the dynamic changes in this receptor that occur as levels of steroid hormones vary may reflect a regulatory feedback mechanism. The hypothesized effect of peripheral benzodiazepine receptor stimulation on the production of neurosteroids also gives rise to prediction that the receptor responds to stressors as part of an integrated compensatory response in which activity at the brain $GABA_A$ receptor complex is ultimately enhanced (Drugan and Holmes, 1991). When assessing the effects of stressors on the peripheral benzodiazepine receptor and the myriad of consequences that results from its activation, our results suggest a need to carefully assess the interaction between the hormonal state of the organism and the specific population of peripheral benzodiazepine receptor under consideration. Such an analysis should clarify the relevance of each of the many systems in which the peripheral benzodiazepine receptor makes a physiological contribution.

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