Pharmacology Biochemistry and Behavior, Vol. 42, pp. 437–444, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Impact of Psychological Dynamics of Stress on the Peripheral Benzodiazepine Receptor

PHILIP V. HOLMES, ANNE P. STRINGER AND ROBERT C. DRUGAN[1]

Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912

Received 9 December 1991

HOLMES, P. V., A. P. STRINGER AND R. C. DRUGAN. *Impact of psychological dynamics of stress on the peripheral benzodiazepine receptor.* PHARMACOL BIOCHEM BEHAV **42**(3) 437–444, 1992.—In an attempt to dissociate the relative impact of psychological vs. physiological concomitants of stress on the peripheral benzodiazepine receptor (PBR), the influence of stressor controllability and predictability was investigated in rats. In addition, the effect of a purely psychological stressor, contextually conditioned fear, was examined. The response of the PBR in rats confronted with a naturalistic threat, a cat, was also tested. Various peripheral and CNS tissues were analyzed. Specific binding of [^{3}H]Ro 5-4864 was significantly reduced in the kidneys of subjects receiving either controllable or uncontrollable shock. Similar changes were seen in the kidneys of subjects receiving either predictable or unpredictable shock. Mean [^{3}H]Ro 5-4864 binding in lung was reduced following both predictable and unpredictable shock, but only the reduction in the predictable shock group reached significance. Controllability appeared to protect against the stress-induced reduction in [^{3}H]Ro 5-4864 binding in lung. Contextually conditioned fear only affected PBR in the olfactory bulb, and exposure to a cat was without effect. These data suggest that the PBR responds only to potent stressors, and psychological influences on the PBR are tissue specific.

Peripheral benzodiazepine receptor Stress Rats Controllability Predictability Conditioned fear

THE central benzodiazepine receptor (CBR) is associated with the GABA type-A receptor/chloride ionophore. This macromolecular complex has been extensively characterized in the CNS (12,14,32). The CBR mediates the anxiolytic, anticonvulsant, muscle relaxant, and sedative actions of benzodiazepines through allosteric interactions with the $GABA_A$ receptor (11,42). That the function of the CBR involves the mediation of fear-related behaviors is supported by evidence of anxiogenic endogenous ligands (1,40) and experiments demonstrating alterations in CBR density (B_{max}) in rats exposed to various stressors (22,44).

Certain benzodiazepines (e.g., valium), the endogenous peptide diazepam binding inhibitor (DBI), and fragments of DBI bind not only to the CBR but also to a distinct type of receptor expressed in a variety of tissues, the peripheral benzodiazepine receptor (PBR) (9,12,36). The PBR is situated primarily on the outer mitochondrial membrane in cells of both the CNS and some peripheral tissues (5). The cDNA encoding the PBR has been cloned, and the apparent translational product of the deduced DNA sequence is a 17- to 18-kD protein (16,41). In the CNS, the PBR appears to be associated primarily with glia (8). The PBR reversibly binds the prototypic ligands Ro 5-4864 and PK 11195 with high affinity (~1 nM). High densities of PBR are found in olfactory bulb, pineal gland, and choroid plexus, as well as the adrenal gland, kidney, lung, and heart (7,8,11,15). Its distinctive subcellular and tissue-specific localization and physical structure have been well characterized, yet its physiological significance is poorly understood.

Recent evidence links the PBR to steroid biosynthesis in adrenocortical and Leydig cells in vitro (9,33,36,37). PK 11195 and Ro 5-4864 are potent stimulators of steroidogenesis whereas lower-affinity PBR ligands such as diazepam are less potent and efficacious (33). Recent work has elucidated a specific role for DBI and a 34 amino acid fragment of DBI, trialkontatetraneuropeptide (TTN), in stimulating steroidogenesis through specific interactions with the PBR (36). The PBR appears to be involved in the delivery of cholesterol through the outer mitochondrial membrane to the side-chain-cleavage enzyme cytochrome P-450 situated on the inner membrane (37,49). This process, in which cholesterol is converted to pregnenolone, is the rate-limiting step in steroidogenesis.

The PBR has also been linked to adrenocorticotropin (ACTH) and corticotropin-releasing hormone (CRH) secretion in vivo and in vitro (13). The molecular mechanisms by which secretion of these peptide hormones is stimulated is unclear. Earlier experiments reported an inhibitory action of Ro 5-4864 on B-endorphin release in vitro, and an interaction

[1] To whom requests for reprints should be addressed.

of PBR ligands with calcium channels in this effect was proposed (10). These actions of PBR ligands on peptide release in vitro require doses at least one order of magnitude higher than the K_d for these compounds, suggesting that such activity does not necessarily depend upon the mitochondrial PBR.

Ro 5-4864 has been reported to be anxiogenic (31) and convulsant (47). However, this action might also involve interactions of the drug with sites other than the PBR. Although in the above studies the CBR antagonist Ro 15-1788 failed to reverse the behavioral actions of Ro 5-4864, there is evidence that Ro 5-4864 may exert some of its effects through interactions with the GABA-gated chloride channel. Ro 5-4864 inhibits its [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding in brain membranes with an IC_{50} value in the micromolar range (43). Further investigations suggested that the interaction of PBR ligands with the TBPS binding site is allosteric. This unique neural Ro 5-4864 binding site may be functionally linked to the site at which various steroid metabolites influence GABAergic transmission (6,23).

Electrophysiological evidence of a biphasic response of Purkinje neurons to PBR ligands in vitro supports the proposal that PBR ligands may exert behavioral effects through activity at both the GABA-gated chloride channel and the mitochondrial PBR (4). In these experiments, Ro 5-4864, PK 11195, and TBPS acted synergistically in producing excitations of cerebellar Pukinje neurons. The slower-developing Ro 5-4864-induced cellular inhibition, on the other hand, was antagonized by PK 11195. The effects of Ro 5-4864 on certain learning and memory tests are dissimilar to those of the anxiogenic CBR ligand FG 7142, further supporting the proposal that the behavioral activity of PBR ligands does not exclusively involve CBR/$GABA_A$ chloride channel mechanisms (24).

Studies of the behavioral actions of PBR ligands are problematic because of the lack of specificity of available compounds. However, an alternative behavioral pharmacology approach provides insight into the functional significance of this receptor system. PBR binding measured in vitro with [^{3}H]Ro 5-4864 or [^{3}H]PK 11195 is altered in tissues from organisms exposed to various environmental manipulations. For example, the density or B_{max} of PBR, like that of CBR, shifts rapidly in rats in response to various stressors. A session of 5 or 80 inescapable tail-shocks increases or decreases, respectively, the binding of [^{3}H]Ro 5-4864 in renal membranes. Cortical PBR binding similarly drops following 80 shocks (18). A brief forced swim increases PBR density in kidney and olfactory bulb (34,39), and the stress of experimental laparotomy alone increases densities in kidney and cortex (35). Alterations in PBR density have also been reported in humans. Maximal [^{3}H]PK 11195 binding is increased compared to controls in the blood platelets of subjects following a stressful examination (25). The PBR thus appears to be involved in an organism's response to stress.

In an attempt to better understand the critical factors influencing the PBR in an organism's stress response, we examined the psychological dimensions and characteristics of a stressor that induces PBR alterations. Our aim was to separate the higher-order neural and more psychological aspects of stress from the metabolic and physical aspects. We also employed a variety of stressors that vary in severity to gauge the relative sensitivity of the PBR response. The central question in the present study was whether the PBR responded to the purely psychological aspects of stress or whether PBR alterations were more reflective of the physiological concomitants of stress. This question arose in part from the initial finding that the renal PBR response to 80 shocks was attenuated by pretreatment with an anxiolytic dose of clonazepam (19). Later work in this laboratory revealed that pretreatment with a sedative/ataxic dose of pentobarbital that does not effect pain sensitivity in the tail-flick test to radiant heat nonetheless attenuates the stress-induced reduction in renal PBR (21).

Using the 80 tail-shock paradigm, we systematically varied the psychological dimensions of controllability and predictability and assessed their possible influence on PBR alterations. Experimental subjects in these paradigms receive the identical physical stress, yet robust behavioral, neurochemical, and physiological differences are observed between those animals that can control or predict the stress and those that cannot (17,26,28,45,46). We also measured the effect of a purely psychological stressor, a contextually conditioned fear paradigm, on the PBR. Finally, we exposed rats to a naturalistic stressor, a cat, to determine whether the PBR is involved in the immediate response to a naturalistic threat.

METHOD

Subjects

Male Sprague-Dawley rats (Charles River, Harlan Laboratory, Brown University-Hunter Laboratory) weighing between 230–300 g at the time of experimentation were group housed (approximately five per cage) with free access to food and water. A 12 L:12 D cycle was maintained in the colony room. Lights were on from 7:00 a.m.–7:00 p.m. Animal experiments were performed between 8:00 a.m. and 12:00 p.m.

Drugs

[^{3}H]Ro 5-4864 (sp. act 79.7 Ci/mM) was purchased from New England Nuclear (Boston, MA). Ro 5-4864 was donated by Dr. Peter Suzdak (Novo/Nordisk Industri, Copenhagen, Denmark) and Dr. Peter Sorter (Hoffman-LaRoche, Nutley, NJ).

Experiment 1: Controllable vs. Uncontrollable Shock

Apparatus. Tail-shocks were administered to rats restrained in Plexiglas escape-yoke wheel-turn boxes (15.5 × 12 × 17 cm). The restraint was accomplished by taping the rat's tail, which protruded through a small notch in the rear of the box, to a 10 × 1 × 1 cm horizontal post extending behind the box. A grooved Plexiglas wheel extended 1.7 cm from the floor of the box. The wheel could be turned by rats in the escape condition to terminate the shock. The wheel in the boxes of yoked animals were immobilized. Shocks were administered by a Lafayette Instruments (Lafayette, IN) Model No. 82400 shock generator through electrodes taped to the tail and augmented with electrode paste.

Procedure. Eighty-three rats were randomly divided into "escape," "yoked," or naive conditions for sacrifice at time points of either immediately or 2 h following stress. Escape and yoked rats received 80 unsignalled tail-shocks (incremented from 1–2 mA) delivered on an average of 1 per min (variable time 60-s schedule). Rats in the escape condition could terminate the shock being administered to both groups by turning the wheel. The two groups thus received the identical amount, intensity, pattern, and duration of shock. If the required number of wheel turns did not occur within a 15-s interval, the shock was automatically terminated. All rats in the present experiment learned the wheel-turn escape response within 20 trials. Rats were sacrificed either immediately or 2 h

following stress and tissues were prepared for in vitro radioligand binding as described below. Naive animals were removed from their home cage and sacrificed at the same time as experimental animals. The order of sacrifice between escape, yoked, and naive animals was counterbalanced.

Experiment 2: Predictable and Unpredictable Shock

Apparatus. The same apparatus as that of Experiment 1 was used with the addition of an MED precision signal generator.

Procedure. Thirty-seven rats were randomly divided into signalled, unsignalled, and naive conditions for sacrifice at time points of either immediately or 2 h following stress. Rats in the signalled condition received a 5-s warning tone (2.8 kHz., 75 dB) prior to the onset of a 5-s shock for a total of 80 shocks administered on a variable time 60-s schedule as described above. Rats in the unsignalled condition received shocks without a warning tone. Shocks were incremented from 1–2 mA over the course of the session as described above. Rats were sacrificed either immediately or 2 h following stress and tissues were prepared for radioligand binding as described below. Naive rats were sacrificed at approximately the same time, and the order of sacrifice was counterbalanced for all animals.

Experiment 3: Contextually Conditioned Fear

Apparatus. A shuttle-box (BRS/LVE, Laurel, MD, Model RSC-004) with inside chamber dimensions of 43 × 20 × 20 cm high equipped with a built-in Sonalert signal generator was used for the contextually conditioned fear paradigm. Shock was delivered to the grid floor of the chamber by a BRS/LVE (Model No. SGS-004) shock generator/scrambler.

Procedure. Sixty-four rats were randomly assigned to one of four groups: "fear conditioned," "tone alone," "no tone," or naive controls for sacrifice either immediately or 2 h following the final conditioned fear stimulus presentation. The experiment thus involved eight groups of rats with eight rats per group. Experimental animals were placed individually into the shuttle-box on day 1 and allowed a 5-min habituation period. The fear-conditioned and no-tone rats received 20 presentations of a 5-s tone followed by a 10-s shock on a variable time 60-s schedule. The tone remained on during the shock and terminated as the shock did (simultaneous conditioning). This procedure was repeated on the following day. The tone-alone group was presented with only the 20 tones and no shock on days 1 and 2. On day 3, following the habituation period, the fear-conditioned and tone-alone groups received five tone presentations on the same schedule (see Table 1 for summary of experimental design). During this time, behavioral observations of freezing time, and the number of crossings in the shuttle-box were recorded as an index of fear. Immediately or 2 h after the last tone presentation, each rat was sacrificed. The no-tone and naive rats were sacrificed directly from the home cage on day 3. The order of sacrifice on day 3 was counterbalanced for group membership.

TABLE 1

EXPERIMENTAL DESIGN FOR CONTEXTUALLY CONDITIONED FEAR

	Treatment		
Group	Day 1	Day 2	Day 3
Fear conditioned	Tone and shock	Tone and shock	Tone
Tone alone	Tone	Tone	Tone
No tone	Tone and shock	Tone and shock	–
Naive	–	–	–

Experiment 4: Exposure to a Cat

Apparatus. The cat was contained in a 75 × 75 × 100 cm steel cage. The spacing between the grates was 3 cm, allowing excellent visibility through the cage. Rats were contained in 20 × 20 × 75 cm plastic tub cages with a steel grate top. The spacing between grates on the rat cage was 1 cm.

Procedure. Twenty-four rats were randomly assigned to the following groups: 15-min cat exposure, 45-min cat exposure, or naive. For the experimental conditions, four rats at a time were placed in a tub cage. The cage containing the cat was then placed directly on top of the rat's cage. Rats were removed from the cat after either 15 or 45 min of exposure and sacrificed. Naive rats were removed from their home cage and sacrificed at approximately the same times for the two experimental groups.

In vitro [^{3}H]Ro 5-4864 Binding

At the appropriate time point following the experimental condition, all rats were sacrificed by decapitation. Tissues were removed and placed in glass vials containing 0.32 M sucrose solution, fast frozen in CO_2/acetone slurry, and stored at −80°C. Tissues were then thawed in a 25°C waterbath, disrupted in 50 vol of 50 mM Tris-HCl buffer (pH = 7.4), and centrifuged at 20,000× g for 20 min. The tissues were resuspended in 200 vol (peripheral tissues) or 100 vol (CNS tissues) of the same buffer, and the binding of [^{3}H]Ro 5-4864 was determined as previously described (48).

Briefly, 0.1 ml peripheral tissue or 0.6 ml CNS tissue (containing ~0.04 or 0.1 mg protein, respectively) was added to each assay tube containing 0.1 ml radioligand (final concentration, 1 nM), 0.1 ml unlabeled drug or buffer, and 50 mM Tris-HCl buffer (pH = 7.4) to a final volume of 1 ml. Assays were performed in triplicate. The reaction was initiated by the addition of tissue and terminated after incubating at 0–4°C for 60 min by rapid filtration over Schleicher (Keene, NH) and Schuell #32 (Keene, NH) glass filter strips using a Brandel (Gaithersburg, MD) M-24R filtering manifold. Samples were washed with two 5 ml aliquots of ice-cold buffer. The specific binding of [^{3}H]Ro 5-4864 was defined as the difference in binding obtained in the presence and absence of unlabeled Ro 5-4864 (final concentration 100 μM). The radioactivity retained by the filters was measured in a Beckman (Beckman Instruments, Fullerton, CA) LS 5000TD liquid scintillation spectrometer using 6 ml ecoscint scintillation solution (National Diagnostics, Manville, NJ) as a fluor. Specific binding accounted for approximately 80–90% of total binding. Protein was determined as previously described (27,30).

Data Analysis

Due to the drift in baseline values typically observed across replications of radioligand binding assays, all data from Experiments 1–3 are presented as percent of mean control values in the concentration of [^{3}H]Ro 5-4864 bound per milligram protein. Since the conversion of raw control values to percent of mean control tends to compress the variability in control groups, all data were analyzed nonparametrically with Mann-

Whitney U-tests. Such analysis allowed direct pair-wise comparisons of experimental to control groups without regard to differences in variance between groups. Percent of mean control values from each experimental group was compared to those of the control groups. In evaluating the significance of the U statistic, the α level of 0.05 was divided by the number comparisons made in each analysis. α Levels for Experiments 1-3 were 0.0125 (four comparisons), 0.025 (two comparisons), and 0.0125 (four comparisons), respectively. This stringent approach was adopted to minimize the probability of Type I error.

Data from Experiment 4 represent a single radioligand binding assay. Baseline drift was therefore not an issue in analyzing these data. Significance was tested by one-way analysis of variance (ANOVA).

RESULTS

Experiment 1: Controllable vs. Uncontrollable Shock

Figure 1 illustrates the effect of escapable and inescapable shock on PBR binding in several tissues. PBR binding immediately following stress was significantly reduced in the kidneys of both groups of subjects receiving escapable and inescapable shock (escape vs. naive: $U = 126.5$, $p < 0.0125$; yoked vs. naive: $U = 139$, $p < 0.0125$). The result of the 80 tail-shock stress paradigm on renal PBR is consistent with the findings of previous experiments (18,19). The dimension of controllability does not appear to influence renal PBR. Two hours following stress, binding in both experimental groups approached control values (escape vs. naive: $U = 111$, $p = 0.024$; yoked vs. naive: $U = 99$, $p = 0.13$). Neither of these U values fall within the rejection region (data not shown).

Unlike the kidney, PBR binding in the lung was differentially influenced by the dimension of stressor controllability. PBR binding in the lungs of rats sacrificed immediately following inescapable shock was significantly reduced compared to naive rats, $U = 203$, $p < 0.0125$. Binding in rats that could escape shock showed no such reduction, $U = 150$, $p = 0.24$. Two hours following stress, both experimental groups exhibited a trend toward reduced binding. The U values, however, did not fall within the rejection region for either group (escape vs. naive: $U = 99$, $p = 0.013$; yoked vs. naive: $U = 99$, $p = 0.013$; data not shown).

PBR binding in heart, adrenal, olfactory bulb, hippocampus, and cortex was not influenced significantly by controllable or uncontrollable stress at either the immediate or 2-h time points.

Experiment 2: Predictable vs. Unpredictable Shock

Both predictable and unpredictable shock caused a significant reduction in renal PBR immediately following stress (see Fig. 2; signalled vs. naive: $U = 15.5$, $p < 0.025$; unsignalled vs. naive: $U = 15.5$, $p < 0.025$). This result represents a direct replication of the effect of 80 5-s tail-shocks on renal PBR (18,19). PBR binding in the lungs of both rats receiving predictable and unpredictable shock were apparently reduced compared to control levels. Only the reduction in PBR binding in lungs of rats receiving predictable shock, however, reached

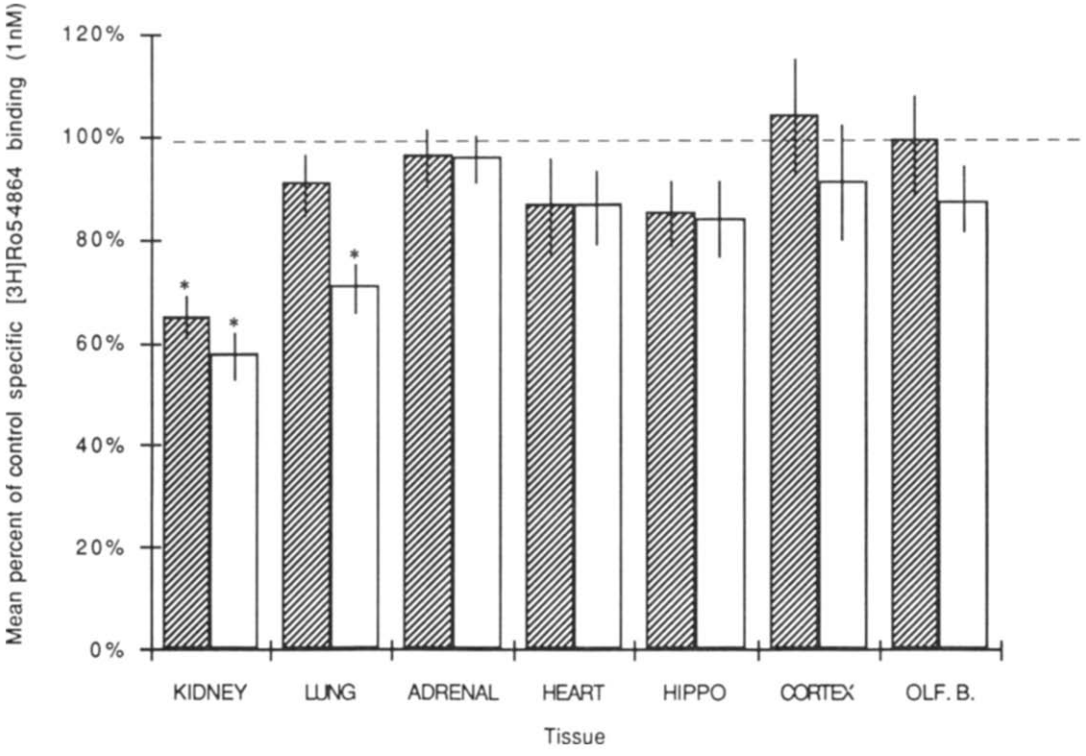

FIG. 1. Impact of stressor controllability on [^{3}H]Ro 5-4864 binding in several rat tissues. Data are presented as mean percent of naive control binding (± SEM). Rats were killed immediately after 80 inescapable or escapable tail-shocks. Open columns represent data from rats receiving inescapable shock. Hatched columns represent data from rats receiving escapable shock. *$p < 0.0125$ compared to naive.

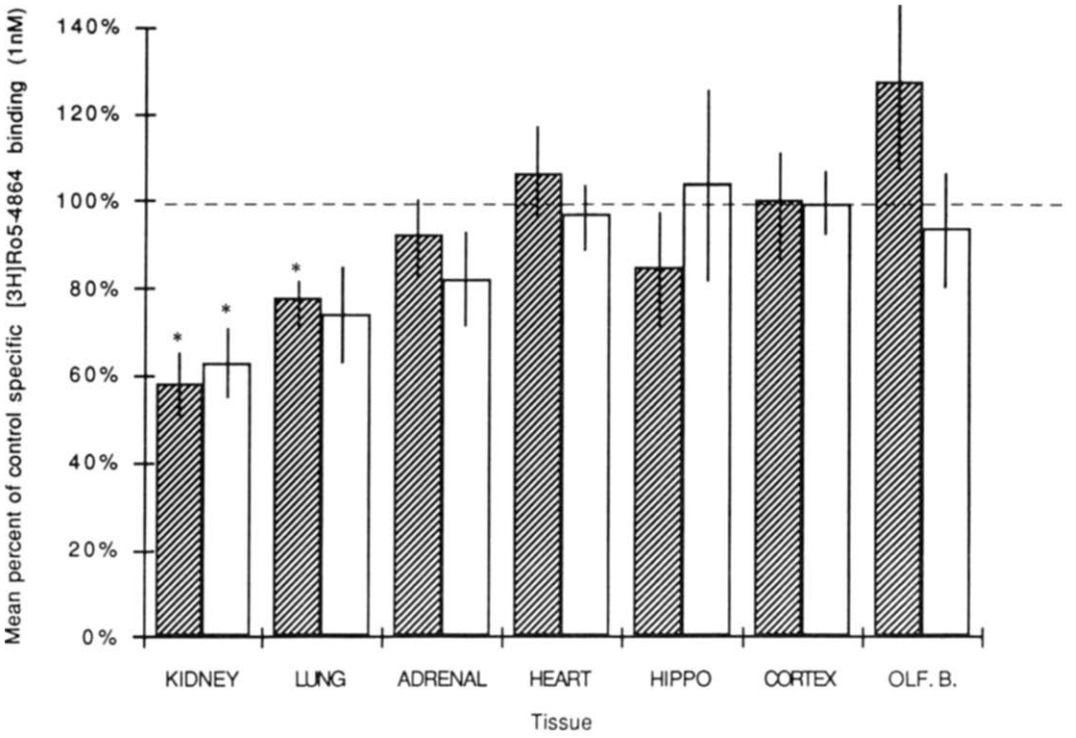

FIG. 2. Impact of stressor predictability on [^{3}H]Ro 5-4864 binding in several rat tissues. Data are presented as mean percent of naive control binding (±SEM). Rats were killed immediately after 80 unsignalled or signalled tail-shocks. Open columns represent data from rats receiving unsignalled shock. Hatched columns represent data from rats receiving signalled shock. *$p < 0.0125$ compared to naive.

significance (signalled vs. naive: $U = 52$, $p < 0.025$; unsignalled vs. naive: $U = 50$, $p < 0.03$).

No significant alterations in PBR binding were observed in heart, adrenal, olfactory bulb, hippocampus, and cortex immediately following predictable or unpredictable shock. Furthermore, no alterations in PBR binding were observed in any tissue at the 2-h poststress time point (data not shown).

Experiment 3: Fear Conditioning

Table 2 illustrates the influence of contextually conditioned fear on the behavioral measures of freezing and locomotor activity (shuttle-box crossings). Presentation of the fear-conditioned tone and context significantly increased freezing (one-way ANOVA: $p < 0.01$) and decreased shuttle-box crossings ($p < 0.01$) compared to rats not aversively conditioned to the tone and context. Behavioral measures thus indicate that fear conditioning was successful. However, the influence of fear conditioning on PBR binding both immediately and 2 h following the procedure was negligible in all tissues surveyed except the olfactory bulb. Figure 3 depicts the PBR binding data at the 2-h time point. PBR binding in the olfactory bulb of fear-conditioned rats was significantly increased compared to the tone-alone control, $U = 56$, $p < 0.0125$. Binding in fear-conditioned rats did not differ from naive rats, $U = 45$, $p = 0.1$. Although trends toward differences between fear-conditioned and control groups were apparent in kidney and hippocampus, these differences were nonsignificant (kidney fear-conditioned vs. naive: $U = 53$, $p = 0.0142$; hippocampus fear-conditioned vs. naive: $U = 45$, $p = 0.03$). These trends may be due to the experimental procedure itself since all experimental groups tended to show increases in [^{3}H]Ro 5-4864 binding irrespective of conditioning. No significant differences in PBR binding were observed in tissues taken from animals sacrificed at the immediate time point (data not shown).

TABLE 2

EFFECT OF CONTEXTUALLY CONDITIONED FEAR ON ACTIVITY IN THE SHUTTLE-BOX ON DAY 3

	Group	
	Fear Conditioned	Tone Alone
% Time freezing*	92 + 0.03†	21 + 0.06
No. of crossings‡	1 + 0.342†	16 + 3.17

*Percentage of time spent immobile, or "freezing," on day 3. Each trial consisted of a 5-min habituation period followed by five tone presentations in the next 4 min. Fear-conditioned rats received context–tone–shock pairing on days 1 and 2. The tone-alone group received only tone presentations. †$p < 0.01$.

‡Mean number of shuttle-box crossings during the 9-min trial session. †$p < 0.01$.

Experiment 4: Exposure to a Cat

No significant alterations in PBR binding were observed in the kidney, heart, lung, adrenal, hippocampus, cortex, or olfactory bulb of rats exposed to a cat for either 15 or 45 min (data not shown).

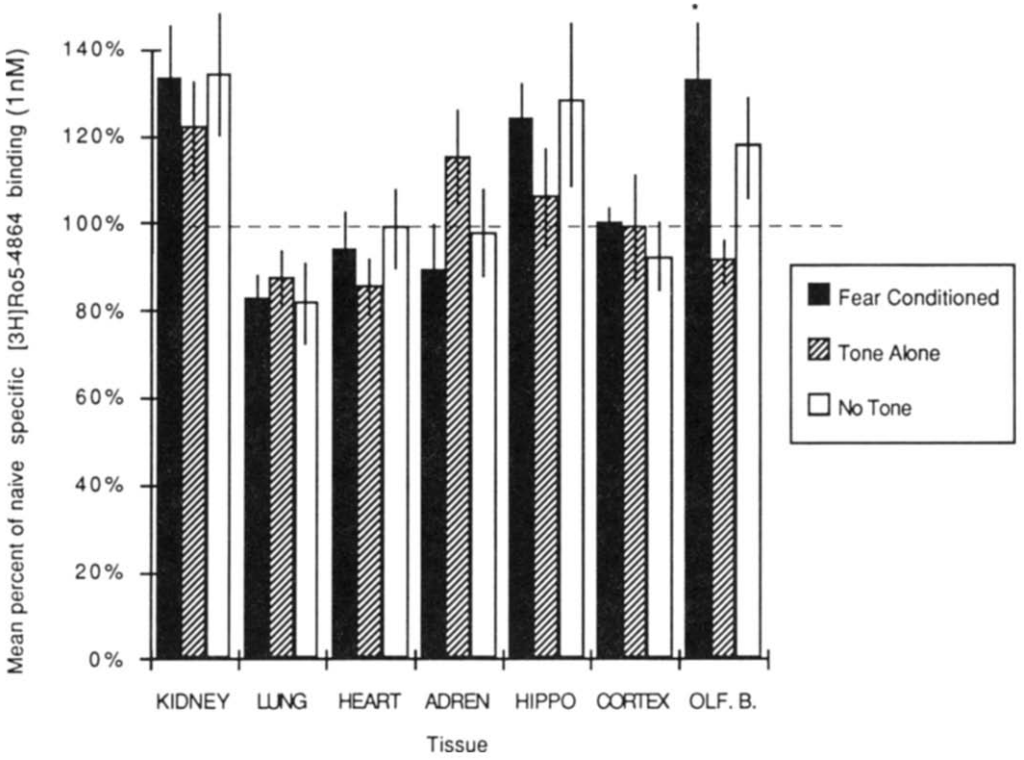

FIG. 3. Effect of contextually conditioned fear on [^{3}H]Ro 5-4864 binding in several rat tissues. Data are presented as mean percent of naive control binding (±SEM). Rats were killed 2 h following the presentation of conditioned stimuli. Shaded columns represent data from fear-conditioned animals. Hatched columns represent data from rats receiving only tones and no shock over days 1-3 ("tone alone"). Open columns represent data from rats receiving tones and shock on days 1-2 and sacrificed from the home cage on day 3 ("no tone"). *$p < 0.0125$ compared to tone-alone control group.

DISCUSSION

These experiments demonstrate that lung and olfactory bulb PBR are responsive to the higher-order, psychological aspects of stress. Renal PBR, on the other hand, responded to stress but were not influenced by the psychological dimensions varied in this study. Although acutely insensitive to psychological manipulations of stress per se, the PBR alteration in kidney is perhaps the most robust and reliable effect reported across different stress paradigms (3,18,34,35,36). The present results suggest that the PBR in kidney, lung, and olfactory bulb are all involved in the stress response but have variant sensitivities to stress. In the 80 shock paradigms, renal PBR may be more dependent upon the broader, purely physiological responses to stress whereas lung PBR may be more influenced by the CNS processes governing differential response strategies to controllable or predictable stress. Lung PBR, however, did not exhibit a consistent response across conditions of controllability and predictability as do other physiological and psychological responses. Stress-induced opiate analgesia, immunosuppression, and learned helplessness, for example, are all averted under conditions of either stressor controllability or predictability (17,26,28,45,46). Stressor controllability appeared to protect against the reduction in lung PBR binding. On the other hand, only the data from subjects receiving predictable stress reached significance. Inspection of the data from the predictability experiment, however, suggests that values in the signalled and unsignalled groups are of the same population. The failure of the unsignalled group to reach significance may be due to the increased variability associated with these values. The narrow region of rejection adopted for the data analysis may thus represent a Type II error in this instance. Although the lung PBR responses differed between the controllability and predictability experiments, it must be pointed out that subjects receive a different amount and pattern of stress in these two paradigms. The duration of shock in the predictability experiment is fixed at 5 s. The duration of shock in the controllability experiment depends upon the wheel-turning response of the subject escaping the shock and may range from approximately 1-15 s within an average duration of 1-2 s once the escape task has been learned.

Unlike renal and lung PBR, olfactory bulb PBR appears to be responsive to a purely psychological stressor. A possible distinction between the role of olfactory bulb PBR and renal and lung PBR might be that of anticipatory stress responses vs. reactive stress responses (20). Olfactory bulb PBR may thus function in a preparatory process; responding to the activation of internal representations of stimuli associated with danger. The conditioned aversive stimuli required to induce PBR alterations in olfactory bulb, however, need to be highly salient, possibly requiring actual physical stress as the unconditioned stimulus (e.g., electric shock). Thus, the olfactory

bulb PBR appears unresponsive to an unconditioned, naturalistic stressor (i.e., confrontation with a cat).

The present experiments support a role of the PBR in an organism's response to stress. This role is evidently complex and poorly understood. As discussed in the introductory section, in vitro studies link the PBR to steroidogenesis in adrenal cells (33,37). Yet, even though the stress paradigms employed in the present experiments enhance steroid release from the adrenal gland (29), the present experiments failed to detect alterations in adrenal PBR. This absence of evidence of a rapid, phasic adrenal PBR response to stress in rats is consistent with previous reports (18). More gradual or tonic adjustments in adrenal PBR following removal of trophic influences (i.e., hypophysectomy) have been reported (2). Individual variations in emotionality may also predict different densities of adrenal PBR (38). PBR response to stress in the adrenal may be too rapid or latent to measure in typical behavioral/stress paradigms. In vivo radioligand binding analysis of the stress-induced changes in PBR should be undertaken to determine whether PBR alterations in some tissues (e.g., adrenal) require factors present only in the intact organism.

The involvement of olfactory bulb, lung, and kidney PBR in the stress response points to other, yet uncharacterized, physiological systems that are activated independently of the pituitary/adrenal axis or sympathetic nervous system (19). The possibility that the PBR in these tissues is involved in the stress-induced synthesis or release of other hormones, neurotransmitters, or autocoids, as well as steroids, deserves further exploration.

In sum, the nature, severity, and time course of stress influence the PBR in a tissue-specific manner. PBR binding in kidney and lung apparently requires relatively severe physical stress, whereas PBR binding in olfactory bulb may be influenced by conditioned stimuli associated with stress. A sensitivity in the PBR response to psychological aspects of stress similar to that of the olfactory bulb has been observed in human tissue. The reported increase in the B_{max} of PBR in human blood platelets following examination stress may be analogous to changes presently reported in the rat olfactory bulb (25). These findings may represent another instance of PBR response to psychological representations of stress and may underlie the earliest stages in a cascade of responses serving to adjust to the physiological demands associated with stress.

ACKNOWLEDGEMENTS

This research was supported by NIMH Grant MH44034 and an Alfred P. Sloan Research Fellowship (BR2852) to R.C.D. All behavioral procedures employed were approved by the Brown University Institutional Animal Care and Use Committee.

REFERENCES

1. Alho, H.; Costa, E.; Ferrero, P.; Fujimoto, M.; Cosenza-Murphy, D.; Guidotti, A. Diazepam-binding inhibitor: A neuropeptide located in selected neuronal populations of rat brain. Science 229:179–182; 1985.
2. Anholt, R. R. H.; De Souza, E. B.; Kuhar, J. J.; Snyder, S. H. Depletion of peripheral-type benzodiazepine receptors after hypophysectomy in rat adrenal gland and testis. Eur. J. Pharmacol. 110:41–46; 1985.
3. Armando, I.; Levin, G.; Barontini, M. Stress increases endogenous benzodiazepine receptor ligand-monoamine oxidase inhibitory activity (tribulin) in rat tissues. J. Neural Trans. 71:29–37; 1988.
4. Basile, A. S.; Bolger, G. T.; Leuddens, H. M. W.; Skolnick, P. Electrophysiological actions of RO5-4864 on cerebellar purkinje neurons: Evidence for "peripheral" benzodiazepine receptor-mediated depression. J. Pharmacol. Exp. Ther. 248:463–469; 1989.
5. Basile, A. S.; Skolnick, P. Subcellular localization of peripheral type binding sites for benzodiazepines in rat brain. J. Neurochem. 461:305–308; 1986.
6. Belelli, D.; McCauley, L.; Gee, K. W. Heterotropic cooperativity between putative recognition sites for progesterone metabolites and the atypical benzodiazepine Ro5-4864. J. Neurochem. 55:83–87; 1990.
7. Benavides, J.; Malgouris, C.; Imbault, F.; Begassat, F.; Uzan, A.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Le Fur, G. Peripheral-type benzodiazepine binding sites in rat adrenals: Binding studies with [^{3}H]PK 11195 and autoradiographic localization. Arch. Int. Pharmacodyn. 266:38–49; 1983.
8. Benavides, J.; Quarteronet, D.; Imbault, F.; Malgouris, C.; Uzan, A.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Le Fur, G. Labeling of peripheral-type benzodiazepine binding sites in the rat brain using [^{3}H]PK 11195, an isoquinoline carboxamide derivative: Kinetic studies and autoradiographic localization. J. Neurochem. 41:1744–1750; 1983.
9. Besman, M. J.; Yanagibashi, K.; Lee, T. D.; Kawamura, M.; Hall, P. F.; Shively, J. E. Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: Stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. Proc. Natl. Acad. Sci. USA 86:4897–4901; 1989.
10. Bisserbe, J. C.; Patel, J.; Eskay, R. L. Evidence that the peripheral-type benzodiazepine receptor ligand Ro5-4864 inhibits B-endorphin release from AtT-20 cells by blockand of voltage-dependent calcium channels. J. Neurochem. 47:1419–1424; 1986.
11. Braestrup, C.; Honore, T.; Nielsen, M.; Petersen, E. N.; Jensen, L. H. Ligands for benzodiazepine receptors with positive and negative efficacy. Biochem. Pharmacol. 33:859–862; 1984.
12. Braestrup, C.; Squires, R. F. Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H]diazepam binding. Proc. Natl. Acad. Sci. USA 74:3805–3809; 1977.
13. Calogero, A. E.; Kamilaris, T. C.; Bernardini, R.; Johnson,E. O.; Chrousos, G. P.; Gold, P. W. Effects of peripheral benzodiazepine receptor ligand on hypothalamic-pituitary-adrenal axis function in the rat. J. Pharmacol. Exp. Ther. 253:729–737; 1990.
14. Costa, E. The supramolecular organization of receptors for gamma aminobutyric acid (GABA). In: Biggio, G.; Costa, E.; Gessa, G. L.; Spano, P. F., eds. Receptors as supramolecular entities. New York: Pergamon Press; 1983:213–235.
15. Davies, L. P.; Huston, V. Peripheral benzodiazepine binding sites in heart and their interaction with dippyridamole. Eur. J. Pharmacol. 73:209–211; 1981.
16. Doble, A.; Benavides, J.; Ferris, O.; Bertrand, P.; Manager, J.; Vaucher, N.; Burgevin, M. C.; Uzan, A.; Gueremy, C.; Le Fur, G. Dihydropyridine and peripheral type benzodiazepine binding sites: Subcellular distribution and molecular size determination. Eur. J. Pharmacol. 119:153–167; 1985.
17. Drugan, R. C.; Ader, D. N.; Maier, S. F. Shock controllability and the nature of stress-induced analgesia. Behav. Neurosci. 99: 791–801; 1985.
18. Drugan, R. C.; Basile, A. S.; Crawley, J. N.; Paul, S. M.; Skolnick, P. Inescapable shock reduces [^{3}H]-Ro5-4864 binding to peripheral type benzodiazepine receptors in the rat. Pharmacol. Biochem. Behav. 24:1673–1677; 1986.
19. Drugan, R. C.; Basile, A. S.; Crawley, J. N.; Paul, S. M.; Skolnick, P. Characterization of stress-induced alterations in [^{3}H]-Ro5-4864 binding to peripheral benzodiazepine receptors in rat

heart and kidney. Pharmacol. Biochem. Behav. 30:1015–1020; 1988.
20. Drugan, R. C.; Holmes, P. V. Central and peripheral benzodiazepine receptors: Involvement in an organism's response to physical and psychological stress. Neurosci. Biobehav. Rev. 15:277–298; 1991.
21. Drugan, R. C.; Holmes, P. V.; Stringer, A. P. Pentobarbital blocks the stress-induced decrease in [^{3}H]Ro5-4864 binding in rat kidney. Brain Res. 535:151–154; 1990.
22. Drugan, R. C.; Morrow, A. L.; Weizman, R.; Weizman, A.; Deutsch, S. I.; Crawley, J. N.; Paul, S. M. Stress-induced behavioral depression in the rat is associated with a decrease in GABA receptor-mediated chloride ion flux and brain benzodiazepine receptor occupancy. Brain Res. 487:45–51; 1989.
23. Gee, K. W. Phenylquinolines PK 8165 and PK 9084 allosterically modulate [^{35}S]t-butlylbicyclophosphorothionate binding to a chloric ionophore in rat brain via a novel RO5-4864 binding site. J. Pharmacol. Exp. Ther. 240:747–753; 1987.
24. Holmes, P. V.; Drugan, R. C. Differential effects of anxiogenic central and peripheral benzodiazepine receptor ligands in tests of learning and memory. Psychopharmacology (Berl.) 104:249–254; 1991.
25. Karp, L.; Weizman, A.; Tyano, S.; Gavish, M. Examination stress, platelet peripheral benzodiazepine binding sites and plasma hormone levels. Life Sci. 44:1077–1082; 1989.
26. Laudenslager, M. L.; Ryan, S. M.; Drugan, R. C.; Hyson, R. L.; Maier, S. F. Coping and immunosuppression: Inescapable but not escapable shock suppresses lymphocyte proliferation. Science 221:568–570; 1983.
27. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
28. Maier, S. F.; Jackson, R. L. Learned helplessness: All of us were right (and wrong): Inescapable shock has multiple effects. Psychol. Learn. Motiv. 13:155–218; 1979.
29. Maier, S. F.; Ryan, S. M.; Barksdale, C. M.; Kalin, N. H. Stressor controllability and the pituitary-adrenal system. Behav. Neurosci. 100:669–674; 1986.
30. Miller, G. Protein determination for large numbers of samples. Anal. Chem. 31:964–967; 1959.
31. Mizoule, J.; Gauthier, A.; Uzan, A.; Renault, C.; DuBroeuque, M.; Gueremy, C.; LeFur, 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.
32. Mohler, H.; Okada, T. Benzodiazepine receptors: Demonstration in the central nervous system. Science 198:849–851; 1977.
33. Mukhin, A. G. Papadopoulos, V.; Costa, E.; Krueger, K. E. Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. Proc. Natl. Acad. Sci. USA 86:9813–9816; 1989.
34. Novas, P. T.; Medina, J. H.; Calvo, D.; DeRobertis, E. Increase in peripheral binding sites in kidney and olfactory bulb in acutely stressed rats. Eur. J. Pharmacol. 135:243–246; 1987.
35. Okun, F.; Weizman, R.; Katz, Y.; Bomzon, A.; Youdim, M. B. H.; Gavish, M. Increase in central and peripheral benzodiazepine receptors following surgery. Brain Res. 458:31–36; 1988.
36. Papadopoulos, V.; Berkovich, A.; Krueger, K. E.; Costa, E.; Guidotti, A. Diazepam binding inhibitor and its processing products stimulate mitochondrial steroid biosynthesis via an interaction with mitochondrial benzodiazepine receptors. Endocrinology 129:1481–1488; 1991.
37. Papadopoulos, V.; Nowzari, F. B.; Krueger, K. E. Hormone-stimulated steroidogenesis is coupled to mitochondrial benzodiazepine receptors: Tropic hormone action on steroid biosynthesis is inhibited by flunitrazepam. J. Biol. Chem. 266:3682–3687; 1991.
38. Rago, L.; Adojaan, A.; Harro, J.: Kiivet, R.-A. Correlation between exploratory activity in an elevated plus-maze and number of central and peripheral benzodiazepine binding sites. Naunyn-Schmied. Arch. Pharmacol. 343:301–306; 1991.
39. Rago, L.; Kiivet, R.-A.; Harro, J.; Pold, M. Central- and peripheral-type benzodiazepine receptors: Similar regulation by stress and GABA receptor agonists. Pharmacol. Biochem. Behav. 32:879–883; 1989.
40. Slobodyansky, E.; Berkovich, A.; Bovolin, P.; Wambebe, C. The endogenous allosteric modulation of $GABA_A$ receptor subtypes: A role for the neuronal posttranslational processing products of rat brain DBI. In: Biggio, G.; Costa, E., eds. $GABA_A$ and benzodiazepine receptor subtypes: Molecular biology, pharmacology and clinical aspects. New York: Raven Press; 1990:51–60.
41. Sprengel, R.; Werner, P.; Seeburg, P. H.; Mukhin, A. G.; Santi, R.; Grayson, D. R.; Guidotti, A.; Krueger, K. E. Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. J. Biol. Chem. 264:20415–20421; 1989.
42. Ticku, M. K.; Maksay, G. Convulsant/depressant site of action at the allosteric benzodiazepine–GABA-receptor–ionophore complex. Life Sci. 33:2363–2375; 1983.
43. Ticku, M. K.; Ramanjaneyulu, R. Ro5-4864 inhibits binding of [^{35}S]t-butylbicyclophosphorothionate to rat brain membranes. Life Sci. 34:631–638; 1984.
44. Trullas, R.; Havoundjian, H.; Zamir, H.; Paul, S. M.; Skolnick, P. Environmentally induced modification of the benzodiazepine/GABA receptor coupled chloride ionophore. Psychopharmacology (Berl.) 97:384–390; 1987.
45. Volpicelli, J. R.; Ulm, R. R.; Alentor, A. A. Feedback during exposure to inescapable shocks and subsequent shock-escape performance. Learn. Motiv. 15:287–292; 1984.
46. Weiss, J. M. Effects of coping behavior in different warning signal conditions on stress pathology in rats. J. Comp. Physiol. Psychol. 77:1–13; 1971.
47. Weissman, B. A.; Cott, J.; Hommer, D.; Paul, S. M.; Skolnick, P. Electrophysiological and pharmacological action of the convulsant benzodiazepine Ro 5-4864. Eur. J. Pharmacol. 97:257–263; 1984.
48. Weissman, B. A.; Skolnick, P.; Klein, D. C. Regulation of peripheral type binding sites for benzodiazepines in the pineal gland. Pharmacol. Biochem. Behav. 21:821–824; 1984.
49. Yanagibashi, K.; Ohno, Y.; Nakamichi, N.; Matsui, T.; Hayashida, K.; Takamura, M.; Yamada, K.; Tou, S.; Kawamura, M. Peripheral-type benzodiazepine receptors are involved in the regulation of cholesterol side chain cleavage in adrenocortical mitochondria. J. Biochem. 106:1026–1029; 1989.