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Environmentally Induced Changes in Peripheral Benzodiazepine Receptors Are Stressor and Tissue Specific

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DRUGAN, R. C., P. V. HOLMES, D. M. SCHER, S. LUCZAK, H. OH AND R. J. FERLAND. *Environmentally induced changes in peripheral benzodiazepine receptors are stressor and tissue specific.* PHARMACOL BIOCHEM BEHAV **50**(4) 551–562, 1995.—The stress-induced changes in peripheral benzodiazepine receptors (PBR) can be observed in a number of different tissues, depending upon the nature and chronicity of the aversive experience. In addition, virtually all stress procedures that cause rapid changes in PBR simultaneously increase the physical activity or metabolic rate of the subjects. The present study analyzed the contributions of rapid alterations in activity or metabolic rate with and without aversive stimulation and their subsequent impact on PBR. Mechanically induced increases in activity by forced running stress results in a significant reduction in [^{3}H]Ro 5-4864 binding to PBR in olfactory bulb, opposite to the PBR changes in this tissue following forced cold-water swim stress. Pharmacological induction of increased locomotor activity as well as metabolic rate by *d*-amphetamine causes a significant increase in cardiac PBR binding, again, opposite to the response typically observed following inescapable shock stress. Finally, administration of the anxiogenic beta-carboline, FG-7142, causes increases in both hippocampus and adrenal gland PBR binding reminiscent of acute noise stress exposure. These experiments demonstrate that increased locomotor activity or metabolic rate alone is not a necessary and sufficient condition for previous stress-induced changes in PBR. Conversely, increased metabolic rate coupled with an aversive stimulus appears to be an important factor for inducing stress-like changes in PBR. This data, coupled with previous reports, suggests that rapid alterations in these sites are stressor and tissue dependent. Finally, we propose that the PBR may be involved in many aspects of the stress response including: a) a biowarning system in adrenal gland, b) participation in stress-induced hypertension via renal PBR, and c) a modulator of stress-induced immunosuppression and subsequent recovery of function or recuperation by actions on immune cells.

Forced running *d*-Amphetamine Beta-Carboline Rat Kidney Heart Adrenal gland
Hippocampus Olfactory bulb Stress

THE PERIPHERAL benzodiazepine receptor (PBR) was discovered simultaneously with the central benzodiazepine receptor (CBR) by two independent groups (64,82). Although great advances have been made regarding the involvement of the CBR in the actions of minor tranquilizers and in the physiological control of anxiety (67,69,83), evidence for the physiological function of the PBR has been lacking until recently (24,25,39,55,86,93). Certain investigators have postulated a role for the PBR in intermediary metabolism (1), synthesis, and/or breakdown of heme and its by-products (87,88), steroidogenesis in both central nervous system (CNS) and peripheral tissue (11,55,56,65,68,73,93), psychoneuroimmune interactions (10,15,17,19,24,28,39,40) and an organism's response to stress (25,28,41,66,92).

Rapid alterations in PBR have been observed. Environmental stress is a potent, phasic regulator of PBR in both CNS and peripheral tissues. Acute exposure to such stressors as inescapable shock (25,27), forced swimming (66,72), noise stress (41,63), conditioned fear (52), and maximal electroshock (9) change the density of PBR in both CNS and peripheral tissue in rats or mice. In humans, examination stress rapidly regulates the density of PBR in blood platelets (54). The

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above changes in the PBR in both rats and humans are quickly initiated and short lived.

The PBR can also be altered in prolonged or chronic states of stress. In rats, genetic selection for high levels of fearfulness (e.g., Maudsley reactive rat) is associated with a reduction in PBR density in both cardiac and renal tissues in comparison to its nonreactive controls (26). Also, rats selected to be "fearful" based on their performance on a plus-maze show changes in PBR density in adrenal gland, kidney, blood platelets, and lymphocytes (70). A human correlate of this chronic anxiety state confirms the observations in these animal models. Patients with generalized anxiety disorder (GAD) have reduced PBR in blood platelets and lymphocytes that is reversed following diazepam treatment (40,74,92). Finally, repeated stress in both animals and humans causes reductions in CNS and peripheral tissue PBRs (22,91).

The localization of the PBR to the outer mitochondrial membrane has been one of the most important clues as to its possible physiological significance (3,8). Due to its subcellular localization, the PBR is thought to be involved in intermediary metabolism (1). A marked density of receptors are found in tissues that derive their metabolic energy primarily from oxidative phosphorylation, while a sparce density of sites is found in tissues that extract their metabolic needs from glycolysis. Histochemical maps showing a significant colocalization between PBR and cytochrome oxidase activity provide strong support for a functional link for these receptors in mitochondrial energy metabolism (3). The mitochondrial localization of the PBR has also prompted the hypothesis and subsequent demonstration of its involvement in other basic physiological processes including steroidogenesis. Prototypical PBR ligands [e.g., Ro 5-4864, PK 11195, and 2-Aryl-3-Indoleacetamide (FGIN-1)] and endogenous substances such as diazepam binding inhibitor (DBI) stimulate pregnenolone formation in various tissues including glial, granulosa, Leydig, and adrenal cells (11,55,56,65,68,73,93). The association of PBR ligands with pregnenalone formation is important because the conversion of cholesterol to pregnenalone is the rate-limiting step in steroid synthesis (81). The interrelationship between PBR, intermediary metabolism, and steroidogenesis suggests a multifaceted role for this receptor in an organism's response to stress (24,28,39).

Many of the stressors used to rapidly modify the PBR confound the presentation of a noxious event with an increase in metabolic rate as well as increased locomotor activity. Yet, no studies have included a systematic analysis of abrupt changes in locomotor activity or metabolic rate alone as important contributors to these stress-induced effects. For example, inescapable shock exposure and forced cold water swim (25,27,66,72) combine noxious exposure (painful or cold exposure/muscle fatigue) with increased metabolic rate (increased heart rate, blood pressure, and oxygen consumption) and gross motor activity (e.g., struggling, movement of all four limbs) in the animals (30,66). Although rapid and sustained increases in locomotor activity can be considered stressful, the nature of this stress is qualitatively different from stressors that are accompanied with aversive or noxious experience. No empirical work to date [see hypothetical comments by (28,29) for exceptions] has entertained the possibility that the increased metabolic rate on the organism or increased horizontal locomotor activity may be sufficient to drive these PBR changes. Indeed, locomotor activity cannot be regarded as a unidimensional concept, so our analysis will be limited to situations or procedures that increase horizontal locomotor activity. Importantly, increased metabolic rate is not inextricably linked to increased motor activity (e.g., anxiety- or fear-induced freezing). Nonetheless, the fact that the PBR is localized to the outer mitochondrial membrane in many tissues, where cellular metabolic activities are performed (47), makes such an empirical analysis all the more crucial. In addition, many of the compounds used to reverse the stress-induced alterations in PBR have an inhibitory effect on both locomotor activity as well as metabolic rate increases associated with stress. For example, the inescapable shock-induced PBR changes are antagonized by tranquilizing or sedating agents such as clonazepam and sodium pentobarbital (27,29), or beta-(4-chlorophenyl-GABA) (72), all of which are known to lower activity, hemodynamic, and oxygen consumption demands induced by stress (48). Thus, in addition to the anxiolytic actions of these compounds, antagonism of the stress-induced increase in metabolic rate or locomotor activity could also be an important factor.

In the present study, we have employed both experimental (forced running) and pharmacological (*d*-amphetamine and beta-carboline administration) methods to produce increases in either locomotor activity or metabolic rate, while independently manipulating aversive properties. This analysis will evaluate stressor and tissue specificity in the rapid environmental alterations in PBR.

EXPERIMENT 1
ACUTE FORCED RUNNING AND RAPID CHANGES IN THE PBR

The inescapable shock procedure used to induce reliable changes in CNS and peripheral tissue PBR is identical to behavioral learned helplessness paradigms in rats (33,53,58). Several studies have examined the activity of rats exposed to inescapable shocks during the pretreatment session. Using ultrasonic motion detectors, we were able to quantify and evaluate the activity of inescapably shocked rats during the initial stress session. The rats exhibit increased activity during the shock presentations, with lower activity during the intertrial intervals. Although the amount of overall activity tends to decrease over the length of the session, this increase in activity over baseline remains sustained throughout the session (30). A forced running procedure, although not identical to the abrupt increases in activity in response to inescapable shock exposure, would, nonetheless, approximate sustained increases in bodily activity as well as metabolic rate typically observed during the stress session, without the presentation of a noxious stimulus.

Acute alterations in PBR have also been reported following 5–15 min of forced swim in cold water (66,72). The tissues showing changes in PBR density include olfactory bulb and kidney. So, in the first set of experiments, we examined the effects of experimentally or pharmacologically induced increases in locomotor activity as well as metabolic stress on PBR binding in these tissues in addition to lung as a control.

Method

Subjects. Twelve male Sprague–Dawley rats obtained from Charles River Laboratories (Wilmington, MA), weighing between 250–300 g, were used as subjects. Prior to and during experimentation all rats were group housed on a standard 12 L : 12 D cycle (lights on at 0600 h) and had free access to lab chow and water. The rats remained in their home cages for 1 week after arrival to allow acclimation to the new environment and avoid any carryover stress effects from shipping.

Apparatus. Animals were run in a wire mesh running wheel made of galvinized steel with a Plexiglas side (Lafayette Instruments Co., Model #86041). The diameter of the wheel was 13.75″ and its width was 4″. In the forced running paradigm, the wheel was driven by an Electro Motor and Control Corp. (Somerville, MA) type NSH-33R motor, 1/20 hp, attached to a Bodine Electric Co. (Chicago, IL) DC motor speed control box. The setting on the box was set to achieve a speed of 8 rpm.

Procedure. Subjects were placed in the motorized running wheel and the motor was turned on for a period of 30 min. The time spent running was recorded on a stopwatch. A separate group of animals, handled controls, were placed in a locked running wheel for the same amount of time as the forced running group.

Immediately following removal from the running wheel, both groups of rats were sacrificed by decapitation. After decapitation, both CNS and peripheral tissue was immediately dissected and placed in glass scintillation vials containing 3 ml of 0.32 M sucrose solution on ice. The tissues were immediately frozen in a solid CO_2/acetone slurry and stored at −80°C until assayed. Radioligand binding of [^{3}H]Ro 5-4864 to PBR in olfactory bulb, kidney, and lung was carried out according to the radioligand binding procedure mentioned below.

In vitro radioligand binding. For all assays in the present article, tissues were removed from the freezer and thawed in a room temperature water bath (25°C). After thawing, the tissues were microdissected and disrupted with a Polytron (setting 6–7, 15 s) in 50 vol of 50 mM Tris-HCl assay buffer (pH = 7.4) and centrifuged at 20,000 × *g* for 20 min at 0–4°C. The pellet was washed and resuspended in either 100 or 200 vol of the same buffer for CNS or peripheral tissue, respectively.

[^{3}H]Ro 5-4864 binding to PBR was determined by the method of Weissman et al. (89). Briefly, 0.1 or 0.6 ml of peripheral or brain tissue (containing approximately 0.04 or 0.1 mg protein, respectively) was added to each assay tube containing 0.1 ml of radioligand (final concentration 1 nM), 0.1 ml of unlabeled drug or buffer and 50 mM Tris-HCl (pH = 7.4) to a final vol 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 and Schull #32 glass filter strips using a Brandel M-24R filtering manifold. Samples were washed with 2–5 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 Instruments LS 5000TD scintillation spectrometer, using 6 ml of Ecoscint (National Diagnostics, Manville, NJ) as a fluor. [^{3}H]Ro 5-4864 (sp. act. 79-85 Ci/mmol) was obtained from New England Nuclear (Boston, MA).

Results

The results of the first experiment are presented in Fig. 1. The acute forced running treatment did not alter the binding of [^{3}H]Ro 5-4864 to PBR in kidney, olfactory bulb, or lung tissue in comparison to naive controls. These observations were confirmed by Student's *t*-tests for each tissue examined. Student's *t*-tests were conducted for both olfactory bulb and kidney tissue because these are the tissues that show rapid,

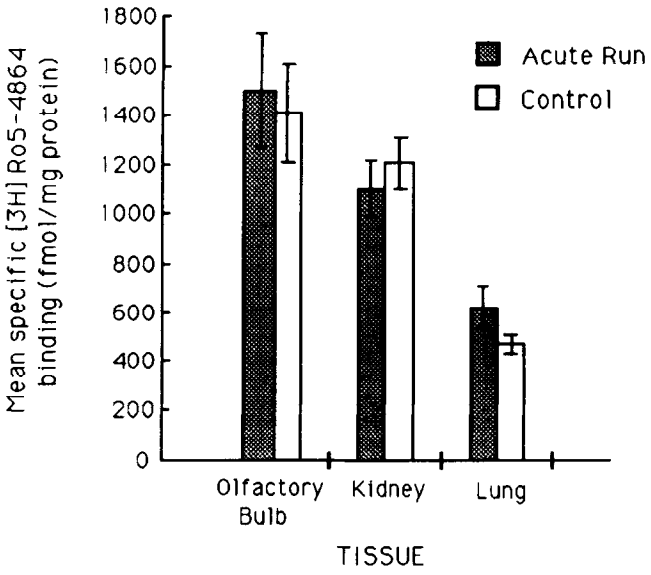

FIG. 1. Mean specific [^{3}H]Ro 5-4864 binding (1 nM) to olfactory bulb, kidney, or lung peripheral benzodiazepine receptors immediately following acute forced running continuously for 30 min on a running wheel or no treatment. Vertical bars represent SEM (n = 6 rats/group).

robust, and reliable alterations following acute swim or shock stress (25,66). For olfactory bulb, the *t*-test indicated a nonsignificant group difference between acute run and controls, $t(10) = 0.29$, $p > 0.1$. Similarly, in renal tissue, no significant group difference was observed, $t(10) = 0.661$, $p > 0.1$.

EXPERIMENT 2
ACUTE AND PROLONGED FORCED LOCOMOTOR ACTIVITY ON A ROTAROD AND SUBSEQUENT CHANGES IN PBR BINDNG

In an effort to examine possible exercise or locomotor activity-induced changes in PBR in both an acute and prolonged situation, we evaluated the changes in PBR in several CNS and peripheral tissues following forced running on a rotarod for either 10 or 80 min. This additional mechanical induction of locomotor activity was employed to provide the capability of evaluating PBR alterations in rats after sustained running for extended periods of time (e.g., 80 min). In the motorized running wheel, the rats were resistant to maintaining sustained running for periods in excess of 30 min. On the contrary, rats will maintain continuous running on a rotarod for periods of time in excess of 1 h (Drugan, unpublished observations).

Method

Subjects. Thirty-two male Sprague–Dawley rats bred and raised at the Walter S. Hunter Laboratory of Psychology at Brown University served as subjects. The rats weighed between 300–400 g at the time of experimentation. All other aspects of housing were identical to those mentioned above.

Apparatus. A Rotarod treadmill (Model #7700) for rats was obtained from UGO Basile Biological Research Apparatus (21025 Comerio, Italy). The rotarod was 35 cm long and was divided into four equal sections by vertical partitions. The cylindrical rod that rotated had a diameter of 6.0 cm, and the speed was adjusted to a constant value of 16 rpms.

Procedure. Subjects were placed on the rotarod and were visually monitored for either the 10- or 80-min session. If the subject fell off the rotarod it was quickly picked up and placed back onto the rod. Poor performance on the rotarod was rarely observed, and did not disrupt the activity of the other animals running simultaneously. The running time of each animal was determined by an LED on the rotarod apparatus. Immediately following the 10- or 80-min session, both experimental and naive rats were sacrificed by decapitation and tissue was rapidly dissected and fast frozen for assay as previously described. Radioligand binding assays were performed on both CNS and peripheral tissue as previously described.

Results

The results of this experiment are shown in Fig. 2. As can be seen in the figure, both acute and prolonged exposure to forced locomotor activity on the rotorod failed to cause changes in PBR binding in either heart, kidney, or adrenal gland. These observations were confirmed by three one-way analysis of variance (ANOVA) in all three analyses, $F(2, 28) < 2.31, p > 0.12$. However, both rotarod conditions resulted in a decrease in olfactory bulb PBR. The acute condition revealed a decrease of 42.7%, while the prolonged condition caused a 48.6% reduction. The significance of these results was confirmed by a one-way ANOVA, $F(2, 28) = 11.12, p < 0.001$. Post hoc Newman-Keuls comparison after ANOVA revealed both rotarod activity conditions were significantly different from the naive group ($p < 0.05$), but did not differ from one another.

EXPERIMENT 3
PHARMACOLOGICAL INDUCTION OF BOTH LOCOMOTOR ACTIVITY AND METABOLIC STRESS ON SUBSEQUENT PBR BINDING: *D*-AMPHETAMINE ADMINISTRATION

Acute or prolonged elevation of locomotor activity by forced running on the rotarod did not alter the binding of [^{3}H]Ro 5-4864 to PBR in either heart, adrenal gland, or kidney. The changes in olfactory bulb were, however, opposite to those observed following cold water swim stress (66,72). Yet, some investigators employ forced running as a stressor itself. Du Ruisseau and colleagues (35) found that forced muscular exercise via a running wheel resulted in significant plasma alterations in several adenohypophyseal hormones including growth hormone, prolactin, follicle-stimulating hormone, thyroid stimulating hormone, and leutenizing hormone. Hence, this experimental procedure may not be an experimentally pure locomotor activity stress and other factors (e.g., occasional falling off the rotarod and handling) may be important contributors as well. The use of pharmacological tools to induce such an increase in locomotor activity as well as metabolic rate would avoid such experimental confounds.

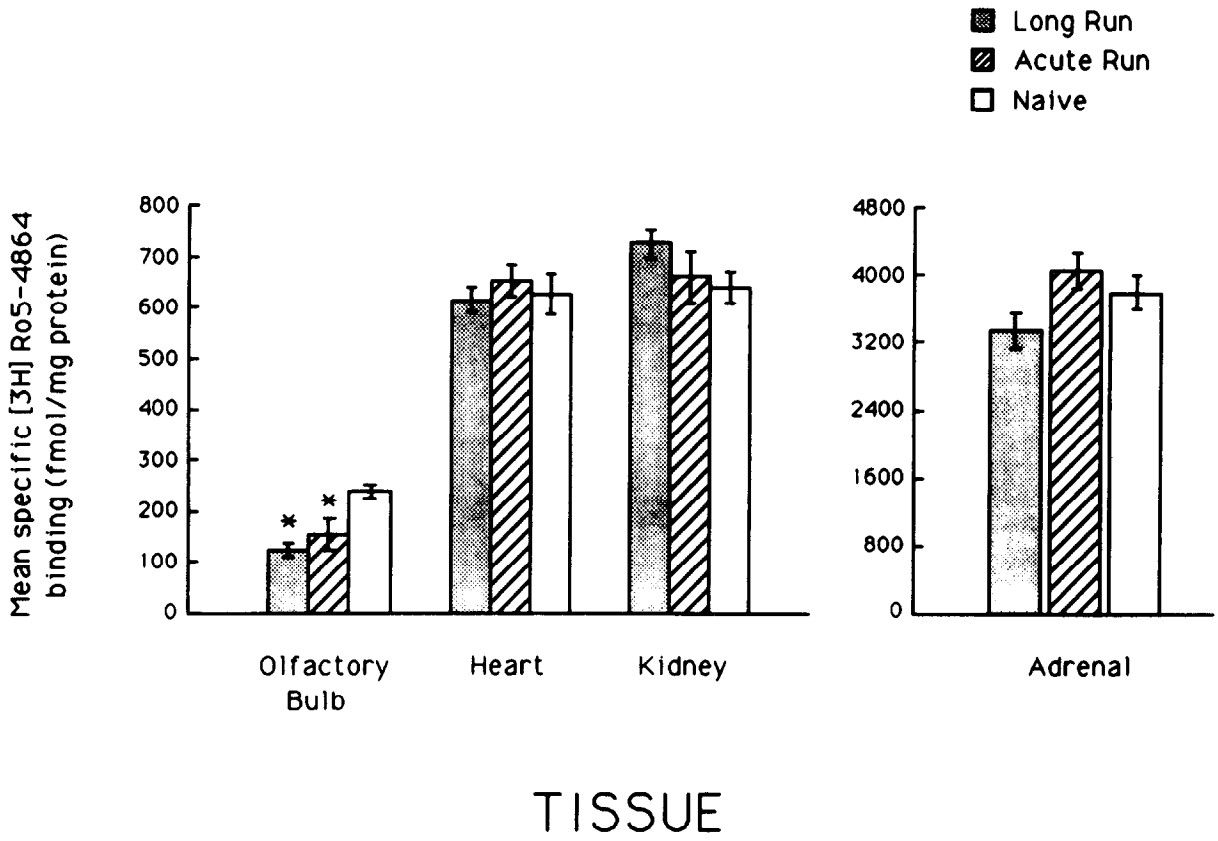

FIG. 2. Mean specific [^{3}H]Ro 5-4864 binding (1 nM) to olfactory bulb, heart, kidney, and adrenal gland peripheral benzodiazepine receptors in rats immediately following acute (10 min) or prolonged (80 min) forced running on a rotorod or no treatment (naive). Vertical bars represent SEM (n = 8–12 rats/group). *Indicates significantly different from naive controls as determined by Newman-Keuls post hoc comparisons after ANOVA ($p < 0.05$).

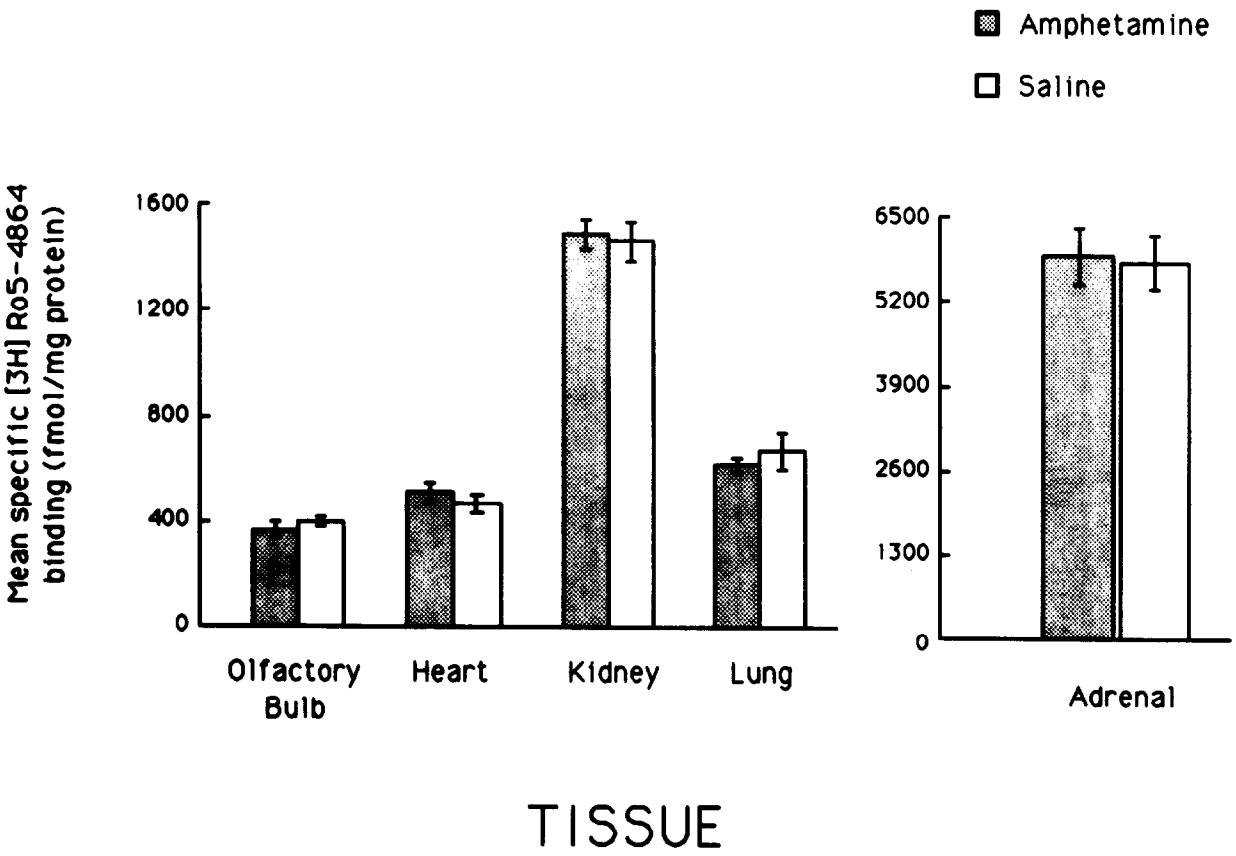

FIG. 3. Mean specific [^{3}H]Ro 5-4864 binding (1 nM) to olfactory bulb, heart, kidney, lung, and adrenal gland peripheral benzodiazepine receptors in rats 25 min following an IP injection of either *d*-amphetamine (2.0 mg/kg) or saline. Vertical bars represent SEM (n = 8 rats/group).

Rapid and sustained increases in metabolic rate as well as locomotor activity without the presence of a noxious agent can be achieved with pharmacological tools. Such agents as methylxanthenes and psychomotor stimulants (e.g., caffeine, theophylline, methylphenidate, and *d*-amphetamine, respectively) potentiate both physical activity and physiological functions such as respiration, metabolic rate, heart rate, and blood pressure (78). However, the use of some of these agents is problematic due to aversive intrinsic behavioral actions that include anxiety (78,79). For this reason, we chose a psychomotor stimulant with opposite (i.e., euphorogenic) actions to the methylxanthenes, *d*-amphetamine. By using an agent that increases locomotor activity and metabolic rate with little or no aversive properties, a rather pure analysis of the contributions of acute alterations in locomotor activity as well as metabolic rate can be assessed.

d-Amphetamine produces a number of behavioral and physiological effects that are dose dependent. In rats, injections of relatively low doses (1.0–3.0 mg/kg, IP) result in at least one component of stereotypy (i.e., continuous sniffing) simultaneous to enhanced locomotor activity (76). Following larger doses (> 5.0 mg/kg, IP), a pattern of behavior is observed that includes a brief increase in locomotor activity that is rapidly replaced by stereotypy (characterized by continuous sniffing, chewing, head swaying, and a lack of rearing or forward locomotion) (76,78,79). We selected a low dose (2.0 mg/kg, IP) of *d*-amphetamine known to produce a maximal amount of locomotor activity when observed up to 2 h postinjection time in rats while minimizing stereotypy (72,78).

As a behavioral confirmation of the efficacy of the 2.0 mg/kg dose of *d*-amphetamine, we conducted a pilot analysis (six rats/group) of rats treated with either 2.0 mg/kg *d*-amphetamine or saline. Twenty minutes following injection, we placed them in a Digiscan open-field apparatus and recorded their vertical and horizontal movements as measured by photocells. We observed a significant and sustained increase in locomotor activity during an 80-min period (data not shown). Our observations were confirmed by a repeated measures ANOVA. The ANOVA indicated a significant treatment (drug vs. vehicle) main effect, $F(3, 30) = 21.97$, $p < 0.001$, and treatment by time interaction, $F(3, 30) = 10.32$, $p < 0.01$, on horizontal locomotor activity measured at four points across an 80-min session. Post hoc Newman-Keuls comparisons after ANOVA ($p < 0.05$) indicated that the amphetamine group differed from the vehicle group at 20, 40, 60, and 80 min postinjection.

Although the above procedure, at best, only approximates the increased activity induced by both shock and swim stress, we feel it is an effective technique for evaluating rapid and sustained increases in locomotor activity as well as metabolic rate on the PBR. The phasic volleys in gross activity, metabolic rate, and repiratory rate observed in an 80-min intermittent inescapable shock session would be very difficult to mimic pharmacologically. We also included an acute amphetamine injection group (rats were sacrificed 25 min following *d*-

amphetamine injection), which would assess the effects of acute increases in locomotor activity induced by 5–15 min of forced swim stress.

Method

Subjects. Thirty-two male Sprague-Dawley-derived rats bred and raised at the Walter S. Hunter Laboratory of Psychology at Brown University were used as subjects. The rats weighed between 250–350 g at the time of experimentation, were group housed (approximately five rats/cage), and had housing conditions identical to the subjects described above.

Apparatus. A Digiscan Animal Activity Monitor (Omnitech electronics Model #RXYZ) was used to perform the open-field activity assessments described in the pilot study.

Procedure. Rats were randomly assigned to one of four groups: acute *d*-amphetamine, acute-saline, prolonged *d*-amphetamine, or prolonged-saline (eight rats/group). The rats in the acute condition received an injection of either *d*-amphetamine (2.0 mg/kg, IP) or saline and were placed back in their home cages. Twenty-five minutes following injection both groups were sacrificed by decapitation and both CNS and peripheral tissues were rapidly dissected, fast frozen and stored at −80°C until assayed. For the prolonged condition, rats received an injection of either *d*-amphetamine or saline as previously described, were placed back into their home cages, and sacrificed 100 min later. Tissues were dissected and frozen for assay as previously described. Although described as a single experiment, the 25- and 100-min amphetamine experiments were conducted successively (i.e., both behavioral and biochemical procedures), so that *t*-test evaluation was conducted rather than an ANOVA.

Results

The 2.0 mg/kg dose of amphetamine did not appear to change the binding of [^{3}H]Ro 5-4864 to olfactory bulb, heart, kidney, lung, or adrenal gland PBR 25 min following injection. The results of 25-min exposure to *d*-amphetamine are illustrated in Fig. 3. These observations were confirmed by Student's *t*-tests in all tissues, $t(14) < 0.99, p > 0.1$.

The effects of 100-min exposure of *d*-amphetamine on PBR binding in both CNS and peripheral tissue are shown in Fig. 4. As can be seen in the figure, exposure to *d*-amphetamine for 100 min failed to cause any changes in PBR in kidney, lung, adrenal gland, or olfactory bulb. However, a noticeable increase in cardiac PBR was evident in comparison to the saline controls. These observations were confirmed statistically in that for all tissues excluding heart the *t*-test value did not approach significance, $t(14) < 1.0, p > 0.2$. However, for cardiac tissue, *d*-amphetamine administration did result in a significant increase, $t(14) = 3.00, p < 0.01$. Interestingly, this effect is in the opposite direction to that seen following prolonged shock stress exposure (i.e., 20% decrease).

EXPERIMENT 4
PHARMACOLOGICAL INDUCTION OF INCREASED METABOLIC RATE ACCOMPANIED BY AVERSIVE PROPERTIES: BETA-CARBOLINE ADMINISTRATION

The previous experiment demonstrated that both acute or prolonged increases in locomotor activity as well as metabolic rate by a nonaversive compound, *d*-amphetamine, was ineffective in producing stress-like changes in the PBR. Perhaps the synergism of metabolic rate increases coupled with an aversive experience are necessary to induce such changes in

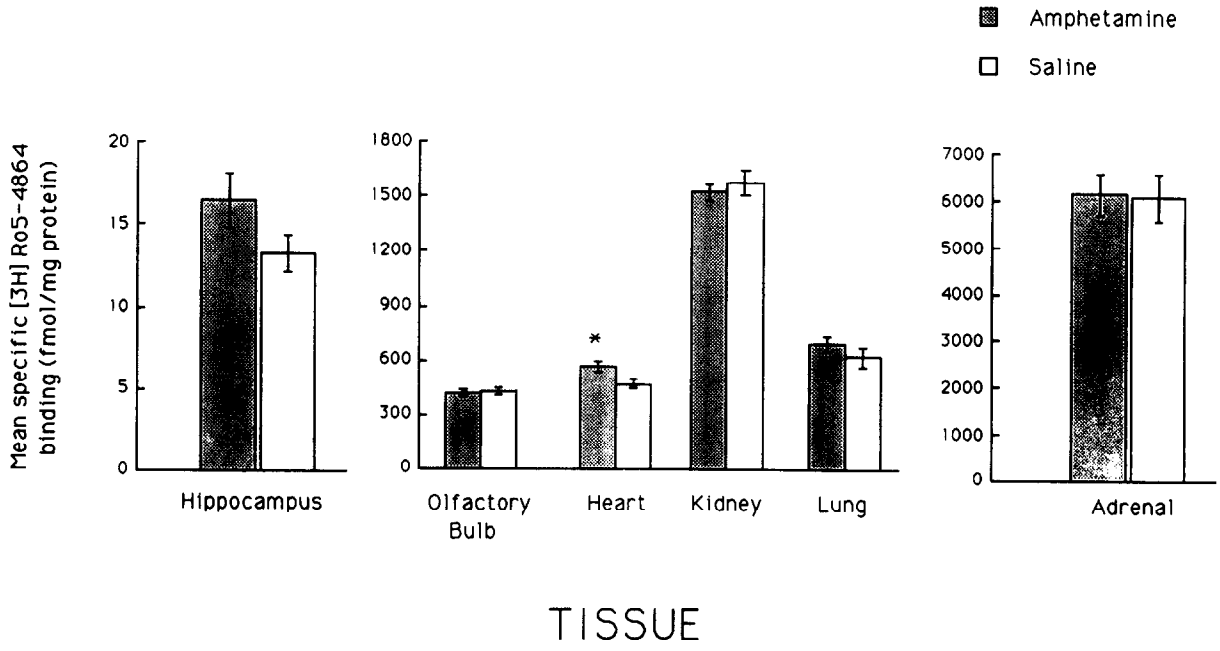

FIG. 4. Mean specific [^{3}H]Ro 5-4864 binding to hippocampus, olfactory bulb, heart, kidney, lung, and adrenal gland peripheral benzodiazepine receptors in rats 100 min following an IP injection of either *d*-amphetamine (2.0 mg/kg) or saline. Vertical bars represent SEM (n = 8 rats/group). *Indicates significantly different from saline control as determined by Student's *t*-test ($p < 0.01$).

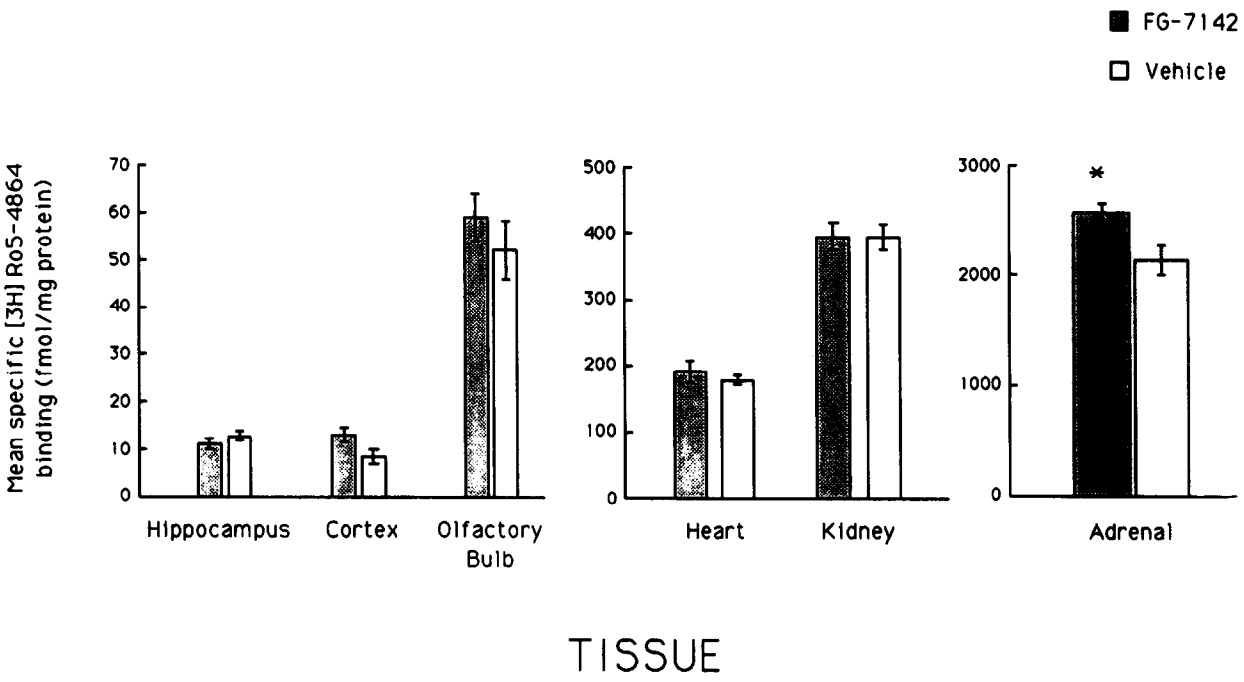

FIG. 5. Mean specific [^{3}H]Ro 5-4864 binding (1 nM) to hippocampus, cortex, olfactory bulb, heart, kidney, and adrenal gland peripheral benzodiazepine receptors in rats 25 min following an IP injection of the beta-carboline, FG 7142 (10 mg/kg) or vehicle. Vertical bars represent SEM (n = 8–18 rats/group). *Indicates significantly different from vehicle control by Student's t-test ($p < 0.05$).

PBR. The advantage of using an anxiogenic beta-carboline, is that one can evaluate the importance of an aversive experience alone, without the confound of pain caused by a physical stressor such as shock. Also, with a beta-carboline, metabolic rate is increased while minimizing locomotor activity due to the behavioral-suppressive effects of this compound (20, 31,62).

The discovery of the anxiogenic compounds beta-carboline-3-carboxylic acid ethyl ester (beta-CCE) and *N*-methyl beta carboline-3-carboxamide (FG-7142) make it possible to study responses to internally induced anxiety (14,20,21,31). In rhesus monkeys, beta-CCE causes rapid changes in metabolic rate characterized by increased heart rate, blood pressure, plasma cortisol, and catecholamine concentrations. Behaviorally, the beta-carbolines cause increases in vocalizations, body twisting, and mouth opening in rhesus monkeys, reflective of intense fear (21). Similarly, FG-7142 administered to human volunteers results in an intense state of anxiety and tension (23). Hence, one can test the necessity of abrupt increases in metabolic rate coupled with an aversive emotional state with these compounds.

Method

Subjects. Thirty-four male Sprague–Dawley rats bred and raised at the Walter S. Hunter Laboratory of Psychology at Brown University were used as subjects. The rats weighed 250–320 g at the time of experimentation and were housed under identical conditions to those described above.

Apparatus. Rats were injected with either drug (FG-7142, Research Biochemicals Incorporated, Natick, MA; 10 mg/kg, IP suspended in distilled water with one drop of Tween-80 per ml) or vehicle solution and placed back into individual cages until sacrifice.

Procedure. Rats were assigned to one of four groups (beta-carboline–25 min, vehicle–25 min, beta-carboline–100 min, vehicle–100 min). The dose of beta-carboline used (FG-7142, 10 mg/kg, IP) was based on previous work from our laboratory and others indicating its efficacy as a potent anxiogenic dose (20,31,62). Twenty-five or 100 min postinjection all rats were sacrificed by decapitation and CNS and peripheral tissues were dissected, fast frozen, and stored at −80°C until assay. Similar to the *d*-amphetamine study, the 25- and 100-min beta-carboline studies were conducted successively, so *t*-test analysis of the data was used instead of ANOVA.

Results

The results of the acute (25 min) beta-carboline exposure is shown in Fig. 5.

As can be seen in the figure, beta-carboline administration for 25 min causes no apparent changes in either CNS or peripheral tissue PBR binding with the exception of adrenal gland. These observations were confirmed with Student's t-tests for all nonadrenal tissues, $t(14) < 2.0, p > 0.1$. However, in adrenal gland, acute beta-carboline exposure resulted in a significant (20.6%) increase, $t(14) = 2.69$, $p < 0.05$. This effect is almost identical to the changes in adrenal gland PBR reported following a brief noise stress in rats (41).

The results of prolonged (100 min) beta-carboline exposure are depicted in Fig. 6.

The figure reveals that no CNS or peripheral tissue PBR binding is altered following this treatment with the exception of hippocampus. These observations were confirmed by Student's t-tests for all nonhippocampal tissues, $t(14) < 2.00$, $p > 0.1$. However, in hippocampus, a significant (27%) increase was observed in the beta-carboline–treated subjects, $t(14) = 2.43$, $p < 0.05$. Again, this beta-carboline–induced

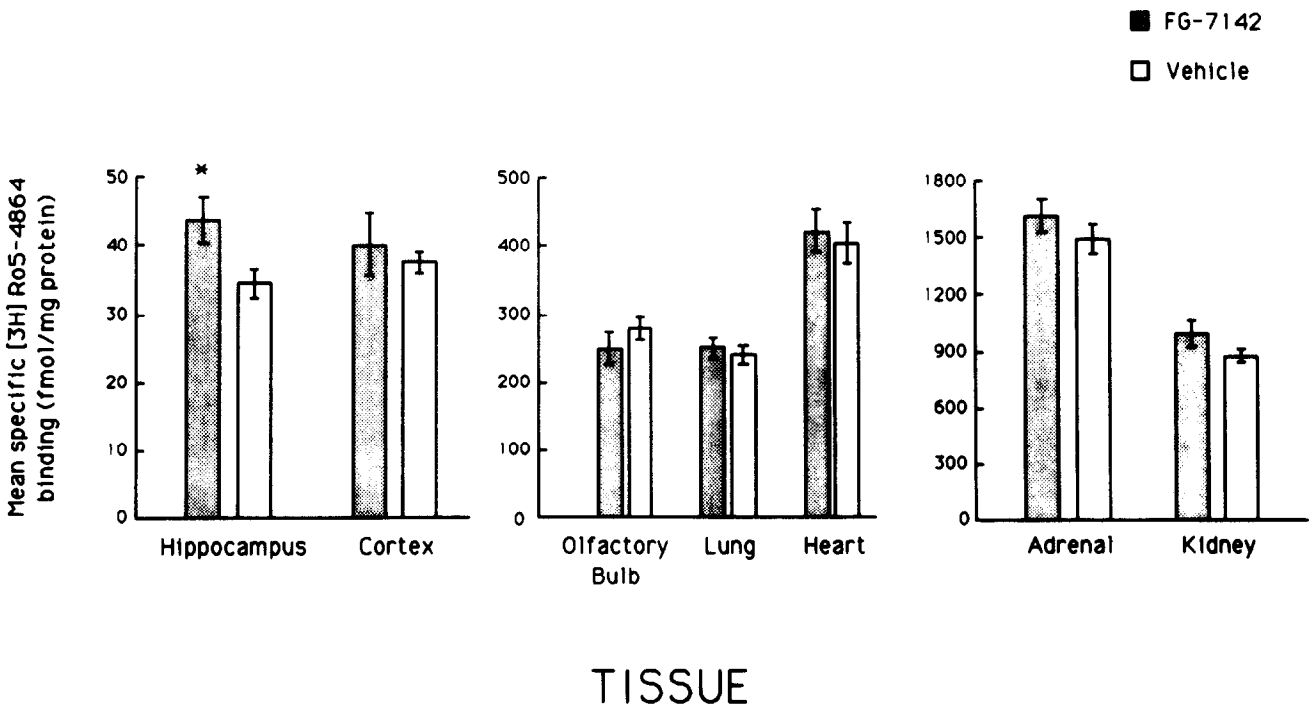

FIG. 6. Mean specific [^{3}H]Ro 5-4864 binding (1 nM) to hippocampus, cortex, olfactory bulb, lung, heart, adrenal gland, and kidney peripheral benzodiazepine receptors in rats 100 min following an IP injection of either the beta-carboline, FG 7142 (10 mg/kg) or vehicle. Vertical bars represent SEM (n = 9–18 rats/group). *Indicates significantly different from the vehicle control by Student's t-test ($p < 0.05$).

change in PBR is similar to the effect produced by noise stress exposure (41).

GENERAL DISCUSSION

The results of the current study provide rather clear evidence for a dissociation between previous stress-induced changes in PBR and abrupt increases in locomotor activity or metabolic rate as possible etiological candidates for these effects. Rapid and sustained increases in locomotor activity or metabolic rate are not sufficient to induce alterations in PBR reminiscent of those seen immediately following either forced-swim or inescapable shock stress. Rather, the synergism of acute increases in metabolic rate coupled with an aversive experience (e.g., anxiety or physical stress) appear to be both necessary and sufficient to cause stress-like changes in PBR.

Acute physically or pharmacologically induced increases in locomotor activity or metabolic rate are ineffective in modulating the PBR in a fashion similar to forced swim or shock in several stress sensitive tissues including olfactory bulb and kidney. These data indicate that the noxious characteristics of the stressor are critical factors in producing rapid changes in PBR. Acute or prolonged elevation of locomotor activity by forced running causes a significnat decrease in olfactory bulb PBR binding, an effect opposite to swim stress. Similarly, prolonged elevation of locomotor activity and metabolic rate by d-amphetamine caused a significant increase in cardiac PBR in comparison to saline controls. However, again this change was opposite to that produced by prolonged inescapable shock exposure. These findings are of interest not only because of the psychomotor stimulant and metabolic properties of this drug, but also its interactions with dopamine systems, suggesting a possible dopamine/PBR connection. Previous work indicates long-term modification of the PBR in both CNS and peripheral tissue following chronic administration of a dopamine antagonist (44–46). However, to our knowledge, the present d-amphetamine-cardiac PBR finding is the first demonstration of a rapid alteration of PBR by a compound that causes the release of catecholamines. Thus, forced running and amphetamine administration can be considered qualitatively different stressors and thereby produce unique changes in PBR in tissues sensitive to these departures from homeostasis.

The increase in metabolic rate coincident with behavioral manifestations of anxiety (e.g., freezing, following beta-carboline administration) caused increased PBR binding in both adrenal gland and hippocampus reminiscent of noise stress exposure (41). This data confirms the critical role of environmentally or pharmacologically induced anxiety/fear in stress-induced changes in PBR (41,52). It is unlikely that the beta-carboline administration caused the respective PBR changes due to direct competitive interactions with [^{3}H]Ro 5-4864 binding at the PBR. First, if indeed a direct influence of beta-carbolines did occur at the PBR, then one would expect to see alterations in a number of tissues sampled, not merely adrenal gland and hippocampus. Second, competitive interactions at the PBR by any compound would be expected to manifest themsleves as a decrease in [^{3}H]Ro 5-4864, while we observed an increase in binding in both tissues. Third, our lab and others have evaluated the competitive nature of a variety of beta-carboline derivatives and have found that these compounds are virtually inactive as inhibitors of [^{3}H]Ro 5-

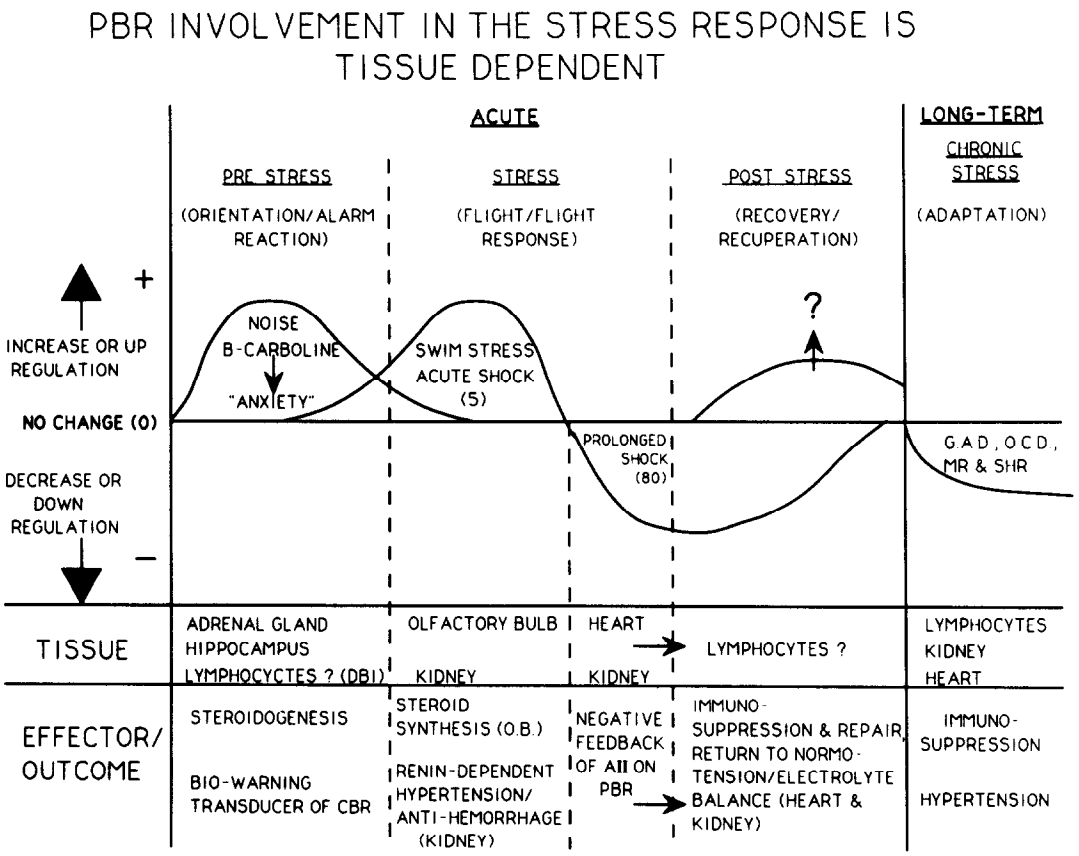

FIG. 7. A theoretical model proposing tissue-selective peripheral benzodiazepine receptor involvement in various stages of the acute stress response as well as in several types of long-term stress or chronic pathologies. (GAD = generalized anxiety disorder, OCD = obsessive-compulsive disorder, MR = maudsley reactive rat, SHR = spontaneously hypertensive rat). Many of the above effects on the PBR can either be induced or alleviated by central benzodiazepine receptor ligands (e.g., beta-carbolines, benzodiazepines, or barbiturates), which gives further support to the notion that these two receptor systems are interrelated in stress reactivity.

4864 binding [i.e., $IC_{50} > 10^{-5}$ M, Drugan, unpublished observations (60,77,89)].

The present results shed light on the nature of PBR reactivity to stress in both CNS and peripheral tissues. Our findings as well as others confirm the initial observation that PBR reactivity to stress is tissue specific (9,25,41,52,63,66,72). Moreover, the stress responsivity of PBR in certain tissues such as adrenal gland and hippocampus appears to be exquisitely regulated, while in other tissues (e.g., heart and kidney) its alteration is driven by much greater physical or noxious demand on the organism. The functional role of the PBR in steroid biosynthesis in various CNS and endocrine tissues becomes especially relevant in light of the present findings coupled with those of Ferrarese et al. (41). The PBR in adrenal gland and hippocampus may be extremely sensitive to alterations in the environment indicating danger or threat to the organism. These new developments necessitate a reevaluation of our original hypothesis (28) stating that PBR changes occur only in reaction to the presentation of a physical stressor. The PBR may be involved in a wide spectrum of changes associated with the stress response, not merely in stress reactivity (albeit in different tissues). Just as Trullas, McIntyre, and Skolnick (86) have proposed the CBR/GABA-A receptor complex in brain as a biowarning system, we propose that the PBR may subserve a similar anticipatory or preparatory function as well. Rapid changes in the brain benzodiazepine/GABA-A chloride ionophore receptor complex are seen within seconds of environmentally threatening stimuli (85). Our rapid, adrenal PBR alterations elicited by a high-affinity anxiogenic brain benzodiazepine receptor ligand, FG-7142, as well as by noise stress (41) may represent the transducing or effector component of this brain biowarning system. The physiological ramifications of this PBR-mediated biowarning system may be enhanced steroid production in a situation of impending danger. This hypothesis is supported by the observation of Ferrarese and colleagues of a correlation between increases in adrenal vein diazepam binding inhibitor (DBI) content, increases in adrenal PBR and increases in plasma corticosterone following noise stress exposure (41). In the present article, we confirm and extend these adrenal PBR findings using a pharmacologically induced state of anxiety. The successive alterations of adrenal gland and hippocampal PBR by both noise stress and beta-carboline-induced anxiety may represent PBR participation in the steroid feedback loop following the stress response. More specifically, the PBR upregulation may either extend or hasten the arrival of nega-

tive feedback by circulating steroids in hippocampal tissue. This putative relationship is currently under investigation.

The acute PBR reactivity in other peripheral tissues such as heart and kidney is not regulated in anticipation of stress. Rather, they are altered once an organism is in contact with a physical stressor and their modification may provide a physiological overlap with the adrenal steroid activation mentioned above. Because the renin-angiotensin system appears to be the critical modulator of the renal PBR stress response (32,49, 50,51), perhaps this system complements the energy-mobilizing effects of the adrenal steroid response by inducing a rapid and sustained condition of hypertension during stress. Just as the outer mitochondrial membrane location of the PBR in adrenal gland prompted systematic study and subsequent demonstration of its involvement in steroidogenesis, this intracellular locale also allows for involvement in the synthesis of hormones other than steroids. Another such hormonal system may be renal eicosanoids. Eicosanoids such as prostaglandins, thromboxanes, and leukotrienes are ubiquitous and potent local factors that modulate cellular activity in a variety of tissues (16,47). Eicosanoids are formed from arachidonic acid, a product of phospholipid metabolism by phospholipase A2. A number of arachidonic acid metabolites are released by endothelial cells in the afferent arteriole that act on the Juxtaglomerular (JG) apparatus to regulate renin release (16). Certain arachidonic acid metabolites including hydroxyeicosatetraenoic acids (HETE) and epoxyeicosatrienoic acids (EET), are formed via arachidonic acid metabolism by cytochrome P-450 (34). Therefore, the link between PBR and the cytochrome P-450 system involved in eicosanoid formation may be similar to that described for steroidogenesis in glial, granulosa, adrenal, and gonadal cell cultures (11,49,55,56,65, 68,73,93). Support for a putative PBR/renin association comes from behavioral pharmacology studies showing a) a significant increase in renal PBR coincident with marked increases in plasma renin activity (PRA) following acute shock stress (25,32), b) the anatomical localization of the stress-induced changes in PBR are primarily localized to the renal cortex, where the JG apparatus resides (39,47) and c) the stress-induced reduction in renal PBR can be blocked with pretreatment of the antihypertensive, angiotensin converting enzyme (ACE) inhibiting agent, captopril (51). The down regulation of PBR following prolonged inescapable shock exposure may represent a negative feedback on renal PBR by high, sustained levels of angiotensin II (AII) during the stress (25,32,49,51). The direct, functional interrelationship between these two systems is currently under investigation.

Finally, recent evidence illustrates a second class of PBRs located on cell membranes of leukocytes in human peripheral blood (10,15,17). This immunological link could provide not only a carrier function of PBR endogenous ligands (e.g., DBI) to a variety of tissues during the initiation of the stress response, but may also provide a novel regulatory mechanism for stress-induced immunosuppression and subsequent recuperation or recovery once the stress has ceased (24,28,39). We have preliminary evidence confirming the functional relevance of the PBR in brain, behavior, and immunity. The PBR antagonist, PK 11195 blocks the inescapable shock-induced suppression of the in vivo antibody response to keyhole limpet hemocyanin (KLH), whereas the CBR antagonist, Ro 15-1788 is without effect (19).

In summary, the apparent chaotic regulation of PBR in a variety of tissues that are differentially activated by stress may, upon further reflection, represent a fine-tuned correlate of the different stages of stress reactivity. This hypothesis is most appealing when viewed in the context of both Cannon (18) and Selye (80) in describing the stress response. Our conceptualization of the PBR in this process is illustrated in Fig. 7.

As can be seen in this model, the PBR may be involved in orienting/alarm, fight or flight as well as recovery of function poststress. Importantly, the participation of the PBR in these varying stages of the stress response depends upon the tissue that is surveyed. Based on such a model, this site may hold promise for pharmacotherapy for a variety of stress-related disorders involving emotional (anxiety), hemodynamic (hypertension), or immunocompetence (immunosuppression or autoimmune diseases) pathologies.

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