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Characterization of Stress-Induced Alterations in [^{3}H] Ro5-4864 Binding to Peripheral Benzodiazepine Receptors in Rat Heart and Kidney

ROBERT C. DRUGAN,*†[1] ANTHONY S. BASILE,† JACQUELINE N. CRAWLEY,* STEVEN M. PAUL* AND PHIL SKOLNICK†

**Clinical Neuroscience Branch, NIMH*
and †Laboratory of Neuroscience, NIDDK
National Institutes of Health, Bethesda, MD 20892

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DRUGAN, R. C., A. S. BASILE, J. N. CRAWLEY, S. M. PAUL AND P. SKOLNICK. *Characterization of stress-induced alterations in [^{3}H] Ro5-4864 binding to peripheral benzodiazepine receptors in rat heart and kidney.* PHARMACOL BIOCHEM BEHAV **30**(4) 1015–1020, 1988.—Inescapable tailshock has been shown to elicit a tissue specific decrease in the density of peripheral benzodiazepine receptors (PBR). We have now explored possible mechanisms that may be responsible for this phenomenon. An 80 minute session of inescapable tailshock produced a reduction in the binding of [^{3}H] Ro5-4864 to renal membranes at 0, 1 and 2 hr after stress, with values returning to control (naive) levels within 24 hr. In cardiac membranes, statistically significant reductions were observed only at 2 and 4 hr after stress. The role of the pituitary-adrenal axis and the sympathetic nervous system in this phenomenon was assessed by subjecting adrenalectomized, hypophysectomized, 6-OHDA-treated or control (sham-operated or saline-treated) rats to inescapable shock. Neither adrenalectomy, hypophysectomy, nor 6-OHDA pretreatment altered the stress-induced reduction in renal PBR. However, the stress-induced decrease in renal PBR was blocked by pretreatment with clonazepam (1 mg/kg), a potent anxiolytic with low affinity for PBR.

Stress Peripheral benzodiazepine receptors Time course Adrenalectomy Hypophysectomy 6-OHDA Clonazepam Heart Kidney

TWO physically and pharmacologically distinct classes of recognition sites for benzodiazepines have been described [26, 30, 31, 34, 36]. "Central" benzodiazepine receptors (CBR), located in tissues derived from the neural crest, are coupled to both a subpopulation of $GABA_A$ receptors and an associated chloride ionophore [32,36]. Both direct and correlative evidence suggests that these sites mediate the principal pharmacological actions of the benzodiazepines, and may also be involved in the physiological response to stress. "Peripheral" benzodiazepine receptors (PBR) are widely distributed in peripheral tissues and are also present in the central nervous system [30, 31, 35]. While neither the physiological nor pharmacological function of PBR are known, recent work has suggested that these sites may be associated with porins [1]. Other studies suggest that PBR are associated with voltage dependent calcium channels [23]. Ro5-4864 (4′-chlorodiazepam), the prototypic PBR ligand, has been reported to possess a number of pharmacologic actions. This compound is a potent convulsant [39,41], potentiates shock-induced suppression of drinking and reduces activity in the social interaction test [17,25]. These actions are similar to those observed following administration of inverse agonists at CBR such as DMCM (methyl-4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate) [14, 19, 27]. While there is good evidence that Ro5-4864 produces its convulsant actions at GABA-gated chloride channels [29, 37, 40], the finding that PK 11195 (a high affinity ligand of PBR that does not interact with GABA-gated chloride channels) [42] can block both the potentiation of shock-induced suppression of drinking and reduction of activity in the social interaction test suggests that some of the behavioral actions of Ro5-4864 may be mediated through PBR. There is additional evidence to suggest that PBR may be involved in stress or anxiety. For example, the Maudsley reactive rat, bred [12] for a high degree of "fearfulness" and increased reactivity to stress, had a significantly lower density of PBR in cardiac and renal

[1]Requests for reprints should be addressed to Robert C. Drugan, Ph.D., Unit on Behavioral Neuropharmacology, Clinical Neuroscience Branch, NIMH, Building 10, Room 4N214, 9000 Rockville Pike, Bethesda, MD 20892.

membranes compared to the Maudsley nonreactive controls [15]. Experimentally-produced stress, such as exposure to inescapable shock, decreased the density of PBR 2 hours postshock in these tissues [16]. In addition, Novas *et al.* [28] have reported that acute exposure to cold water swim stress results in an increase of PBR in both kidney and olfactory bulb. These data suggests that the PBR in certain peripheral organs, such as heart and kidney, may be altered during stress. These findings are supported by the observation that PBR on platelet membranes are significantly reduced in patients with generalized anxiety disorder (in comparison to age- and sex-matched controls), and normalized by diazepam [43].

As an extension of our previous work demonstrating tissue-specific changes following exposure to inescapable shock, we have further characterized the nature of these changes by examining: (1) the time course for their development, (2) the possible hormonal and sympathetic nervous system modulation of these effects, and (3) the effect of a centrally-active anxiolytic with low affinity for PBR on these changes.

METHOD

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200–300 g received no treatment (naive) or were restrained in Plexiglas wheel-turn boxes (15.5×2×17 cm) modeled after those used by Weiss *et al.* [38]. A grooved Plexiglas wheel extended 1.7 cm into the front of the chamber through a hole 8.0 cm from the floor of the box. The rat's tail was extended through a slot in the rear wall of the chamber and was taped to a Plexiglas rod parallel to the floor of the chamber. Shock generators (Layfette Instruments, Model No. 82400) were used to apply 80 unsignalled inescapable shocks (incremented from 1–2 mA) lasting five seconds and delivered through electrodes attached to the tail. The shocks were presented on a variable time schedule on an average of 1 per minute.

For analysis of the time course, rats were killed by decapitation following no treatment (naive) or at 0, 1, 2, 4 and 24 hours after the last shock. As specified, male Sprague-Dawley rats (150–175 g) were either adrenalectomized, hypophysectomized, or sham operated at Taconic Farms (Germantown, NY). Following all surgical manipulations, the experimental protocol consisted of placing the operated and sham surgery rats in either an experimental (shock) or untreated (naive) condition. The adrenalectomized rats were evaluated 1 week following surgery, while hypophysectomized rats were tested 4 weeks postsurgery. The 6-OHDA animals were tested 1 week following the last of 2 injections of 200 mg/kg IP which were separated by 1 week. Finally, in the clonazepam study, rats were injected with clonazepam (1 mg/kg) 30 minutes prior to the experiment. Animals were sacrificed 2 hours following the last shock except in those experiments evaluating the time course. Tissues were immediately removed and placed in an isotonic sucrose solution (0.32 M), fast frozen in a solid CO_2/acetone slur, and stored at −80°C until use to ensure optimal assay conditions [3].

Radioligand Binding Assays

In all experiments, the tissues were thawed in a water bath at 50°C. Immediately upon thawing the tissue was then homogenized using a Brinkman Polytron (setting 6–7, 15 seconds) in 50 volumes of 50 mM Tris-HCl buffer (pH 7.4)

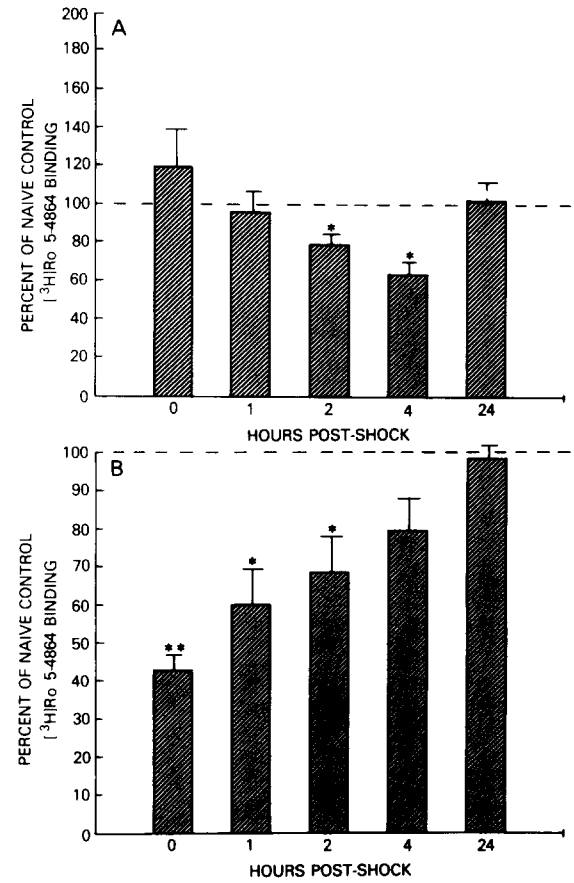

FIG. 1. Although each time point was run and assayed at a different time, the data are presented in the same figure for ease of inspection. The data are presented as the mean (n=4/group) percent of naive control [^{3}H] Ro5-4864 binding (10 nM) and were analysed by individual Student's *t*-tests because the baseline naive control binding values do vary significantly from experiment to experiment. Panel A reveals significant reduction in [^{3}H] Ro5-4864 binding to cardiac PBR was observed 2 (19%) and 4 (33%) hours postshock [$t(6)=2.44$, $p<0.05$; and $t(6)=1.87$, $p<0.05$, one-tailed test]. In contrast, no significant differences in [^{3}H] Ro5-4864 binding to cardiac PBR were observed at 0, 1, or 24 hr after shock. Panel B reveals that a significant reduction in the binding of [^{3}H] Ro5-4864 to kidney PBR is observed at 0 (57%), 1 (39%), and 2 (31%) hr postshock, $t(6)=5.55$, $p<0.01$; $t(6)=2.70$, $p<0.05$; $t(6)=2.36$, $p<0.05$, while at 4 and 24 hr postshock no significant differences were observed. **Indicates significantly different from naive controls, $p<0.01$; *$p<0.05$.

and centrifuged at 20,000×g for 20 minutes. The pellets derived from heart and kidney were resuspended in 400 volumes of buffer. The [^{3}H] Ro5-4864 binding was determined as described by Weissman *et al.* [39]. Briefly, 0.1 ml of peripheral tissue (containing ~0.02 mg protein) was added to each assay tube containing 0.1 ml of radioligand (final concentration 10 nM), 0.1 ml of unlabelled drug or buffer, and buffer 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 (0–4°C) for 60 min by rapid filtration over Whatman GF/B strips using a Brandel M-24R filtering manifold. Samples were washed with two 5 ml aliquots of ice-cold buffer. The specific binding of [^{3}H] Ro5-4864 was defined as the difference in binding obtained in the

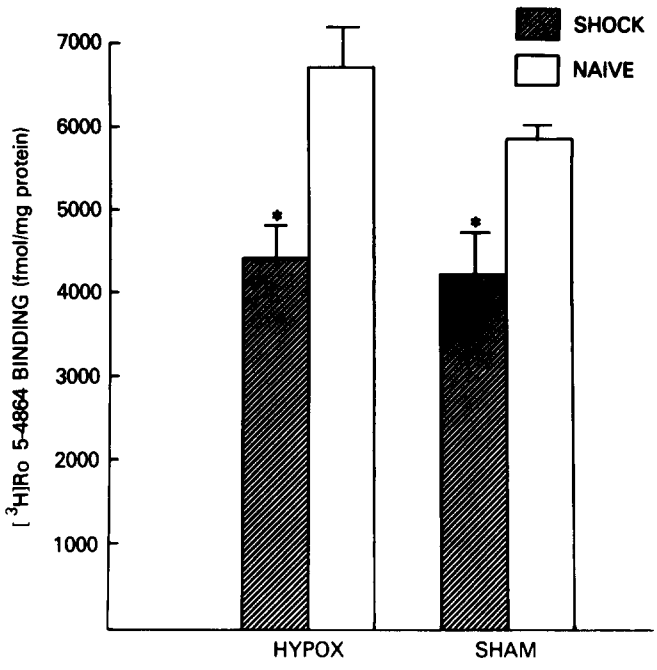

FIG. 2. Mean (n=8/group) specific [^{3}H] Ro5-4864 binding (10 nM) to renal PBR of hypophysectomized or sham-operated rats 2 hours following inescapable shock or no treatment (naive). A two-way analysis of variance revealed a significant treatment (shock) main effect, $F(1,28)=18.16$, $p<0.001$, while the surgery (hypox vs. sham) main effect and treatment × surgery interaction were not significant. Subsequent Newman-Keuls comparisons ($p<0.05$) revealed that both the hypox-shock and sham-shock groups were significantly different from their respective stress-naive controls which did not differ from each other. *Indicates significantly different from naive controls by Newman-Keuls ($p<0.05$) individual comparisons after ANOVA.

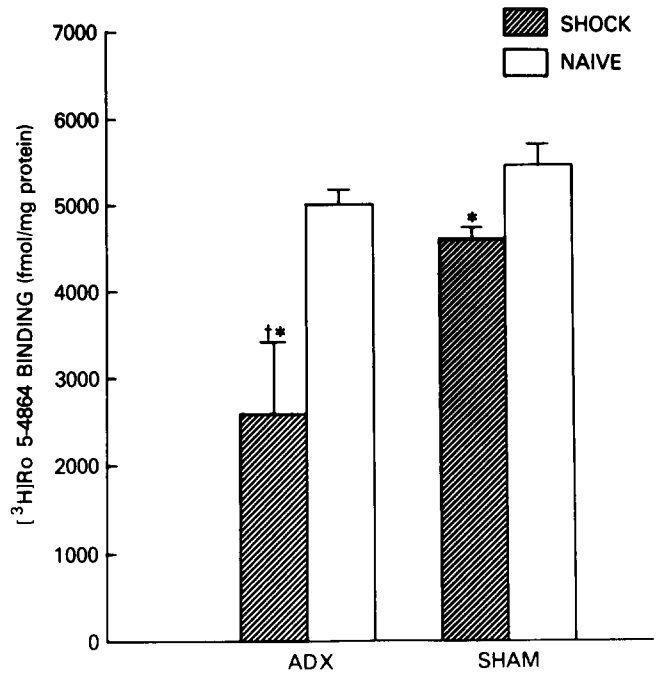

FIG. 3. Mean (n=5/group) specific [^{3}H] Ro5-4864 binding (10 nM) to renal PBR of adrenalectomized or sham-operated rats 2 hours following inescapable shock or no treatment (naive). A two-way analysis of variance revealed a significant treatment (shock) main effect, $F(1,16)=17.51$, $p<0.001$, and a significant surgery (ADX vs. sham) main effect, $F(1,16)=11.20$, $p<0.01$. Subsequent Newman-Keuls comparisons ($p<0.05$) revealed that both ADX-shock and sham-shock groups were significantly different from their respective controls. Finally, the ADX-shock group differed significantly from the sham-shock group. *Indicates significantly different from naive controls by Newman-Keuls ($p<0.05$) individual comparisons after ANOVA. †Indicates significantly different from sham-shock.

presence and absence of unlabelled Ro5-4864 (final concentration, 5 μM). The radioactivity retained by the filters was measured in a Beckman LS 5801 liquid scintillation spectrometer, using 5 ml of Ready-solv MP (Beckman Instruments, Fullerton, CA) as a fluorophore. [^{3}H] Ro5-4864 (sp. act 81.5 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Ro5-4864 was a gift of Hoffmann-LaRoche, Nutley, NJ. Protein was determined using the Miller [24] modification of the method of Lowry *et al.* [21].

RESULTS

Time Course

Significant decreases of 22% and 35% were observed in cardiac membranes 2 and 4 hours postshock. Values returned to control (i.e., baseline) levels by 24 hours (Fig. 1A). In renal membranes, significant decreases (58%) were observed immediately after shock, one hour (40%) and 2 hours (32%) postshock. Values returned to control (i.e., baseline) levels by 24 hours (Fig. 1B).

Effects of Hypophysectomy

The effect of hypophysectomy on the inescapable shock-induced reduction in [^{3}H] Ro5-4864 binding to renal PBR is shown in Fig. 2. As can be seen, both the hypophysectomized and sham-operated rats displayed similar reductions in the binding of [^{3}H] Ro5-4864 to renal PBR.

Effects of Adrenalectomy

The effect of adrenalectomy on the inescapable shock-induced reduction in [^{3}H] Ro5-4864 binding to renal PBR is shown in Fig. 3. In confirmation of previous findings [4] no significant alteration in [^{3}H] Ro5-4864 binding to renal PBR is observed one week following adrenalectomy. The sham-operated rats that were exposed to inescapable shock showed a reduction (15%) in the binding of [^{3}H] Ro5-4864 to renal PBR. Adrenalectomized rats exposed to inescapable shock displayed a much larger reduction (48%) in [^{3}H] Ro5-4864 binding to renal PBR in comparison to sham-shocked rats.

Effects of 6-Hydroxydopamine Injections

The effects of 6-OHDA or saline injections on the inescapable shock-induced decrease in [^{3}H] Ro5-4864 binding to renal PBR are shown in Fig. 4. 6-OHDA injections did not alter the binding of [^{3}H] Ro5-4864 to renal PBR in naive rats nor did it affect the reduction in PBR produced by inescapable shock.

Effects of Clonazepam

The effects of clonazepam administration (1 mg/kg) 30

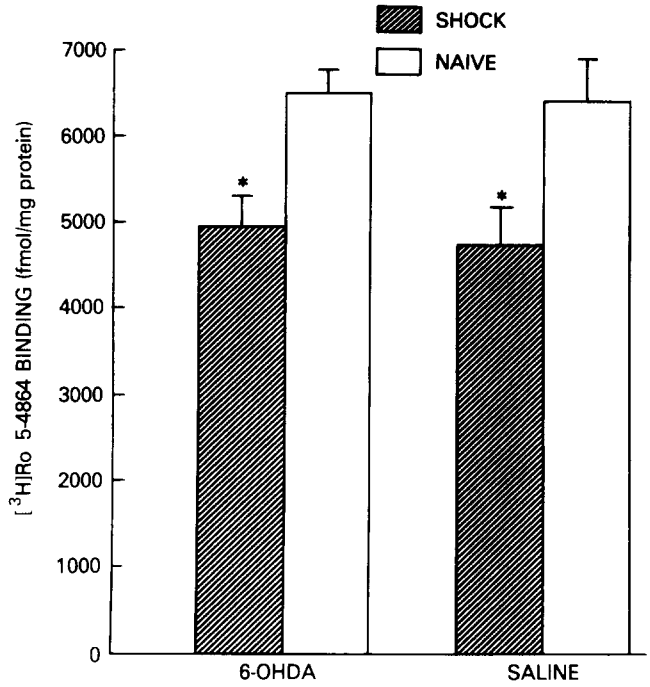

FIG. 4. Mean (n=8/group) specific [^{3}H] Ro5-4864 binding (10 nM) to renal PBR of 6-OHDA- or saline-treated rats 2 hr following inescapable shock or no treatment (naive). A two-way analysis of variance revealed a significant treatment (shock) main effect, $F(1,28)=15.81$, $p<0.001$. Subsequent Newman-Keuls comparisons ($p<0.05$) indicated that both 6-OHDA-shock and saline-shock groups were significantly different from their naive controls which did not differ from one another. *Indicates significantly different from both naive controls by Newman-Keuls individual comparisons after ANOVA.

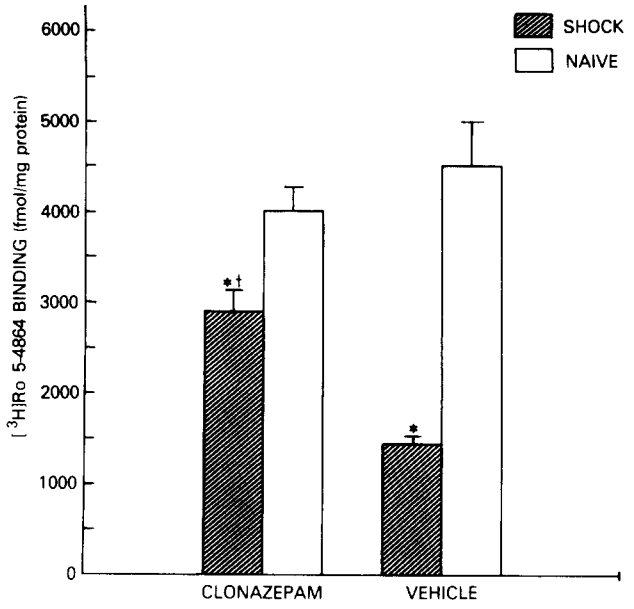

FIG. 5. Mean (n=4/group) specific [^{3}H] Ro5-4864 binding (10 nM) to renal PBR of rats given either clonazepam (1 mg/kg, IP) or saline 30 minutes prior to a session of inescapable shock or no treatment (naive). A two-way analysis of variance revealed a significant treatment (shock) main effect, $F(1,12)=38.72$, $p<0.001$, and a significant drug × treatment interaction, $F(1,12)=7.92$, $p<0.01$. Subsequent Newman-Keuls comparisons ($p<0.05$) indicated that the vehicle-shock group was significantly different from the vehicle-naive group, while the clonazepam-shock group was also significantly different from the clonazepam-naive group. Nonetheless, [^{3}H] Ro5-4864 binding to renal PBR of the clonazepam-shock group was significantly higher than the vehicle shock group while the clonazepam-naive and vehicle-naive groups were not different. *Indicates significantly different from respective control group, †indicates significantly different from vehicle shock group by Newman-Keuls comparisons ($p<0.05$) after ANOVA.

minutes prior to a session of inescapable shock are shown in Fig. 5. Rats injected with vehicle prior to inescapable shock showed a dramatic reduction (~66%) in the binding of [^{3}H] Ro5-4864 to renal PBR compared to vehicle-naive rats. However, administration of clonazepam prior to inescapable shock attenuated this shock-induced decrease by 50%. Thus, there was a significant difference between the clonazepam and vehicle shock groups (Fig. 5).

DISCUSSION

Our previous studies [15,16] have shown that the changes in renal PBR resulting from 80 minutes of inescapable tail-shock are manifested as a decrease in the maximum number of binding sites (β_{max}) for [^{3}H] Ro5-4864 with no change in the apparent affinity (K_d) of this radioligand. The present study demonstrates that the inescapable shock-induced decrease in [^{3}H] Ro5-4864 binding to cardiac and renal PBR produced by inescapable shock is both rapid and short-lived. In cardiac membranes, the stress-induced decrease in [^{3}H] Ro5-4864 binding was observed 2 and 4 hours postshock but returned to control levels within 24 hours. A decrease in [^{3}H] Ro5-4864 binding to renal PBR was observed immediately following the 80 minute shock session and displayed a rapid return to baseline. Recently, Novas *et al.* [28] reported that exposure to acute stress results in an increase in renal PBR which appears to be at variance with our current findings. However, we have demonstrated that the magnitude and direction of changes in the binding of [^{3}H] Ro5-4864 may be dependent on such factors as the duration and intensity of the stressor [16].

A genetic predisposition for heightened reactivity to stress as well as exposure to environmental stressors such as inescapable shock are both accompanied by changes in hormonal and neurotransmitter systems thought to be intimately involved in an organism's response to stress. For example, the Maudsley reactive rat exhibits significantly higher heart rate, blood pressure, basal serum prolactin, brain stem levels of serotonin, hypothalamic dopamine and concomitant lower norepinephrine levels in hypothalamus, heart, spleen and adrenals compared to Maudsley nonreactive controls [7–10, 20, 33]. In addition, exposure to inescapable shock results in a significant activation of the pituitary-adrenal axis as evidenced by significant increases in both plasma corticosterone and adrenocorticotrophic hormone (ACTH) levels [22]. Hence, the changes in PBR density observed in the Maudsley reactive rat and in rats exposed to inescapable shock could be modulated by hormones and/or activation of the sympathetic nervous system. These findings, coupled with recent studies which have demonstrated that the density of PBR are under hormonal control [2, 4, 18], prompted our

study of the role of the sympathetic nervous system and the pituitary-adrenal axis in this effect. Hypophysectomy did not alter the effect of inescapable shock on renal PBR, suggesting that pituitary trophic hormones and/or peptides are not responsible for these stress-induced changes. Adrenalectomy potentiated the stress-induced reduction in renal PBR. Although no significant changes in renal PBR density were observed 1 week following adrenalectomy (Basile *et al.* [4] and the present paper), a synergistic action of adrenalectomy and inescapable shock may be responsible for the potentiation, suggesting that adrenal steroids or mineralcorticoids may be important in this effect. Throughout these studies, we observed that prior stress such as sham-surgery appeared to attenuate the subsequent inescapable shock-induced decrease in renal PBR. Analysis of variance confirmed this observation of a significant difference between the levels of [^{3}H] Ro5-4864 binding to renal tissue in shocked rats for all experiments, $F(3,21)=7.84$, $p<0.01$. However, there was also a significant variance in the levels of [^{3}H] Ro5-4864 binding to renal tissue in naive rats for all experiments, $F(3,21)=3.39$, $p<0.05$, demonstrating that intraexperimental variability contributed to these apparent differences.

Alterations in the sympathetic nervous system have been shown to affect the density of PBR in certain tissues. Weissman *et al.* [42] have shown that constant light exposure or superior cervical ganglionectomy, experimental manipulations that alter sympathetic innervation to the pineal gland, produced a significant decrease (~50%) in the density of PBR in this organ. We investigated the importance of the sympathetic nervous sytem on stress-induced changes in renal PBR by treating rats with either 6-OHDA (200 mg/kg, IP) or saline prior to inescapable shock. The 6-OHDA regimen that we employed destroys 88–92% of the catecholaminergic input to peripheral tissues (such as kidney) 7 days after treatment [5]. Chemical sympathectomy (via systemic 6-OHDA injections) did not influence the stress-induced changes in renal PBR, suggesting that catecholamines or changes in sympathetic outflow is not a critical factor.

Our hypothesis that PBR density changes are stress or anxiety related was supported by the observation that clonazepam (1 mg/kg, IP) given prior to inescapable shock significantly attenuated the stress-induced decrease in [^{3}H] Ro5-4864 binding to renal PBR. The benzodiazepine clonazepam was chosen because of its low affinity for PBR ($Ki>>1$ μM) relative to CBR (K_d 3 nM) [35]. Thus, it is likely that the attenuation of the shock-induced decrease in renal PBR can be considered to be a result of an anxiolytic action of the CBR rather than a direct effect at the PBR. This finding suggests the neurobiological concomitants of stress and anxiety play an important role in the regulation of renal PBR.

REFERENCES

1. Anholt, R. R. H. Mitochondrial benzodiazepine receptors as potential modulators of intermediary metabolism. Trends Pharmacol. Sci. 7:506–511; 1986.
2. Anholt, R. R. H.; De Souza, E. B.; Kuhar, M. 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. Basile, A. S.; Skolnick, P. Preservation of "peripheral-type" benzodiazepine binding sites: differential effects of freezing on [^{3}H] Ro5-4864 and [^{3}H]PK 11195 binding. J. Pharmacol. Methods, in press; 1987.
4. Basile, A. S.; Ostrowski, N. L.; Skolnick, P. Aldosterone reversible decrease in the density of renal peripheral benzodiazepine receptors in the rat after adrenalectomy. J. Pharmacol. Exp. Ther. 240:1006–1013; 1987.
5. Basile, A. S.; Skolnick, P. Tissue specific regulation of "peripheral-type" benzodiazepine receptor density after chemical sympathectomy. Life Sci.; in press.
6. Basile, A. S.; Paul, S. M.; Skolnick, P. Adrenalectomy reduces the density of "peripheral-type" binding sites for benzodiazepine in the rat kidney. Eur. J. Pharmacol. 110:149–150; 1985.
7. Blizzard, D. A.; Hansen, C. T.; Freedman, L. S. Open-field behavior and the peripheral sympathetic nervous system in the MR/N and MNR/N rat strains. Behav. Genet. 12:459–466; 1982.
8. Blizzard, D. A.; Liang, B.; Emmel, D. K. Blood pressure, heart rate and plasma catecholamines under resting conditions in rat strains selectively bred for differences in response to stress. Behav. Neural Biol. 29:487–492; 1980.
9. Blizzard, D. A.; Liang, B. Central serotonergic function and behavior in the Maudsley reactive and nonreactive strains: A reevaluation. Behav. Genet. 9:413–418; 1979.
10. Blizzard, D. A.; Slater, J.; Liang, B.; Shenkman, L. Serum prolactin and hypothalamic dopamine in rat strains selectively bred for differences in susceptibility to stress. Neuroendocrinology 23:279–305; 1977.
11. Braestrup, C.; Schmeichen, R.; Neef, G.; Nielsen, M.; Petersen, E. Interaction of convulsant ligands with benzodiazepine receptors. Science 216:1241–1243; 1982.
12. Broadhurst, P. L. Experiments in personality. In: Eysenck, H. J., ed. Psychogenetics and psychopharmacology. vol. 1. London: Routledge and Keegan Paul Company; 1969.
13. Corda, M.; Blaker, W.; Mendelson, W.; Guidotti, A.; Costa, E. β-carbolines enhance the shock-induced suppression of drinking in the rat. Proc. Natl. Acad. Sci. USA 80:2072–2078; 1983.
14. Dorow, R.; Horawski, R.; Paschelke, G.; Amin, M.; Braestrup, C. Severe anxiety induced by FG-7142, A beta-carboline ligand for benzodiazepine receptors. Lancet 11:98–99; 1983.
15. Drugan, R. C.; Basile, A. S.; Crawley, J. N.; Paul, S. M.; Skolnick, P. "Peripheral" benzodiazepine binding sites in the Maudsley reactive rat: Selective decrease confined to peripheral tissues. Brain Res. Bull. 18:143–145; 1987.
16. 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.
17. File, S. E.; Lister, R. G. The anxiogenic action of Ro5-4864 is reversed by phenytoin. Neurosci. Lett. 35:93–96; 1983.
18. Gavish, M.; Weizman, A.; Okun, F.; Youdim, M. B. H. Modulatory effects of thyroxine treatment on central and peripheral benzodiazepine receptors in the rat. J. Neurochem. 47:1106–1110; 1986.
19. Insel, T. R.; Ninan, P. T.; Aloi, J.; Jimerson, D. C.; Skolnick, P.; Paul, S. M. A benzodiazepine receptor-mediated model of anxiety. Arch. Gen. Psychiatry 41:741–750; 1984.
20. Liang, B.; Blizzard, D. A. Central and peripheral norepinephrine concentrations in rat strains selectively bred for differences in response to stress: Confirmation and extension. Pharmacol. Biochem. Behav. 8:75–80; 1978.
21. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193:265–275; 1951.
22. 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.

23. Mestre, M.; Carriot, T.; Belin, C.; Uzan, A.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Doble, A.; LeFur, G. Electrophysiological and pharmacological evidence that peripheral type benzodiazepine receptors are coupled to calcium channels in the heart. Life Sci. 36:391–396; 1985.
24. Miller, G. Protein determination for large numbers of samples. Anal. Chem. 31:964; 1959.
25. Mizoule, J.; Gauther, A.; Uzan, A.; Renault, C.; Dubroeucq, M.; Gueremy, C.; LeFur, G. Opposite effects of ligands for peripheral type binding sites, PK 1195 and Ro5-4864, in a conflict situation in the rat. Life Sci. 36:1058–1068; 1985.
26. Mohler, J.; Okada, T. Benzodiazepine receptors: Demonstration in the central nervous system. Science 198:849–851; 1977.
27. Ninan, P. T.; Insel, T. R.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. Benzodiazepine receptor mediated anxiety in primates. Science 218:1332–1334; 1983.
28. Novas, M. L.; Medina, J. H.; Calvo, D.; De Robertis, E. Increase of peripheral type benzodiazepine binding sites in kidney and olfactory bulb in acutely stressed rats. Eur. J. Pharmacol. 135:243–246; 1987.
29. Obata, T.; Yamamura, H. I. Inhibition of GABA-stimulated chloride influx by the convulsant benzodiazpeines Ro5-3663 and Ro5-4864 into membrane vesicles from rat cerebral cortex. Eur. J. Pharmacol. 136:447–448; 1987.
30. Shoemaker, H.; Bliss, M.; Yamamura, H. I. Specific high affinity saturable binding of [^{3}H] Ro5-4864 to benzodiazepine binding sites in the rat cerebral cortex. Eur. J. Pharmacol. 71:173–175; 1981.
31. Shoemaker, H.; Boles, R. G.; Horst, D.; Yamamura, H. I. Specific high-affinity binding sites for [^{3}H] Ro 5-4864 in rat brain and kidney. J. Pharmacol. Exp. Ther. 225:61–69; 1983.
32. Skolnick, P.; Paul, S. M. Benzodiazepine receptors in the central nervous system. Int. Rev. Neurobiol. 23:103–136; 1978.
33. Slater, J.; Blizzard, D. A.; Pohorecky, L. A. Central peripheral norepinephrine metabolism in rat strains selectively bred for differences in response to stress. Pharmacol. Biochem. Behav. 6:511–520; 1977.
34. Squires, R.; Braestrup, C. Benzodiazepine receptors in rat brain. Nature 266:732–734; 1977.
35. Syapin, P.; Skolnick, P. Characterization of benzodiazepine binding sites in cultured cells of neural origin. J. Neurochem. 32:1047–1051; 1979.
36. Tallman, J. F.; Paul, S. M.; Skolnick, P.; Gallager, D. W. Receptors for the age of anxiety: the pharmacology of benzodiazepines. Science 207:274–281; 1980.
37. Ticku, M. K.; Ramanjaneyulu, R. Ro5-4864 inhibits the binding of [^{35}S] t-butylbicyclophosporothionate to rat brain membranes. Life Sci. 34:631–638; 1984.
38. Weiss, J. M.; Stone, E. A.; Harrell, N. Coping behavior and brain norepinephrine levels in rats. J. Comp. Physiol. Psychol. 72:153–160; 1970.
39. Weissman, B. A.; Bolger, G. T.; Isaac, L.; Paul, S. M.; Skolnick, P. Characterization of the binding of [^{3}H] Ro5-4864, a convulsant benzodiazepine, to guinea pig brain. J. Neurochem. 42:969–975; 1984.
40. Weissman, B. A.; Cott, J.; Jackson, J. A.; Bolger, G. T.; Weber, K. H.; Horst, W. D.; Paul, S. M.; Skolnick, P. "Peripheral-type" binding sites for benzodiazepines in brain: relationship to the convulsant actions of Ro 5-4864. J. Neurochem. 44:1494–1499; 1985.
41. Weissman, B. A.; Cott, J.; Paul, S. M.; Skolnick, P. Ro5-4864: a potent benzodiazepine convulsant. Eur. J. Pharmacol. 90:149–150; 1983.
42. 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.
43. Weizman, R.; Tanne, Z.; Granek, M.; Karp, L.; Golomb, M.; Tyano, S.; Gavish, M. Peripheral benzodiazepine binding sites of platelet membranes are increased during diazepam treatment of anxious patients. Eur. J. Pharmacol. 138:289–292; 1987.