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BRIEF COMMUNICATION

β-Adrenergic Receptor Changes in Learned Helplessness May Depend on Stress and Test Parameters

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BRANNAN, S. K., A. MILLER, D. J. JONES, D., G. L. KRAMER AND F. PETTY. *β-Adrenergic receptor changes in learned helplessness may depend on stress and test parameters.* PHARMACOL BIOCHEM BEHAV 51(2/3) 553-556, 1995.—Behavioral deficits following inescapable stress (learned helplessness) may serve as an animal model of depression. Previous studies using foot-shock stress to induce learned helplessness and a bar-press test for the stress-induced behavioral deficit have found increased β-adrenergic receptor density in the hippocampus of learned helpless rats. We replicated these experiments using a tail-shock stress and the shuttle-box test. In our experiments, rats that developed learned helplessness after inescapable stress did not demonstrate any significant differences in β-adrenergic receptor density or affinity in the frontal cortex, cerebellum, or hippocampus compared to the nonhelpless rats, nor to the tested control rats. These results suggest that β-adrenergic receptor changes in learned helplessness may depend on the specific stress and test procedures used.

β-Adrenergic receptor Learned helplessness Depression Norepinephrine Animal models

ANIMAL studies have consistently found behavioral deficits to occur in a proportion of animals exposed to inescapable stress (12). Stress-induced behavioral deficits include subsequent deficient responding in tests with escapable stress, termed *learned helplessness* (LH) (7,15). This phenomenon has been proposed as an animal model of human depression. Behavioral signs of depression modeled by LH include reduced motor activity, reduced appetite behavior, deficits in grooming behavior, and disturbance of sleep (32). LH can be prevented or reversed by antidepressant drugs (25,28), suggesting that the model may also have pharmacologic relevance.

One of the major neurochemical theories of depression involves dysregulation of the noradrenergic system. A major and consistent laboratory finding in support of the noradrenergic theory of depression is that chronic antidepressant treatments decrease the density of β-adrenergic receptors in limbic forebrain, including hippocampus of the rat (6). In the LH animal model, an increased β-receptor density in the hippocampus of helpless rats has been reported (8,16). Because decreased β-receptor density in this region is produced by chronic antidepressant treatments, increased β-receptor density in the hippocampus may be a neurochemical correlate of the behavioral signs of depression. These experiments induced LH with foot-shock stress, and then were tested for behavioral depression with a bar-press escape task.

Another procedure for studying LH in the rat involves using inescapable tail-shock stress and a shuttle-box test (12).

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If the correlation between biochemistry and behavior for an animal model is robust, it should generalize, and not be contingent upon, the specific test used. Therefore, we hypothesized that increased β-receptor density would be found in hippocampus of helpless rats, using tail-shock stress to induce LH and a shuttle-box test for subsequent behavioral depression.

METHOD

Learned helplessness induction and testing in these experiments were as previously described (5,23). Male Wistar rats (Sasco Inc., Indianapolis, IN) weighing 275–300 g at the time of the experimentation, were used. Briefly, animals were exposed to 80 1-s unsignaled inescapable shocks (1.0 mA increased by 0.2 to 0.3 mA in blocks of 20 shocks to 2.0 mA, with a 60-s variable ITI) delivered via electrodes attached to the tail while the rats were confined to plastic chambers. Twenty-four hours after inescapable tail-shock stress, rats were tested for LH. Testing consisted of five fixed response one (FR1) trials followed by 25 FR2 trials. Each trial began with he onset of 1.0-mA scrambled foot-shock, which was terminated by shuttling or after 30 s if no escape occurred. A 60-s variable ITI separated trials. Based on data from nonstressed tested rats, an FR2 trial with escape latency > 20 s was defined as an escape failure (9). Animals exposed to this paradigm that demonstrated mean escape latencies for the FR2 trials of > 20 s were defined as LH (5). Animals with escape latencies < 20 s were defined as nonlearned helpless (NLH). Both mean escape latency and number of escape failures are given. A group of rats that were not exposed to tail-shock but received the shuttle-box test served as the control group. Thus, there were three experimental groups: stressed rats that developed (LH) or failed to develop (NLH) learned helplessness, and nonstressed tested controls. All rats were euthanized 24 h after testing, corresponding to the time of maximum increased β-adrenergic density previously reported (8). Following decapitation, brain regions were rapidly dissected on ice and frozen at −70°C.

The frozen brain regions were thawed, homogenized, and assayed for β-adrenergic receptors using the ligand [^{125}I]-iodocyanopindolol ([^{125}I-ICYP) via filtration assay (20). Briefly, prepared tissue homogenates were incubated for 45 min at 37°C at nine different concentrations (20–600 pM) of [^{125}I]-ICYP. Nonspecific binding was defined by 1 μm of DL-propranolol. Protein was assayed with the method of Lowry et al. (11).

Saturation isotherms were analyzed by using a nonlinear least squares solution of ligand binding parameters for calculation of B_{max} and K_d (Lundon Software Inc., Chatrin Falls, OH). All binding data were analyzed by two-way analysis of variance (ANOVA) followed by comparison of least square means. Behavioral data were analyzed with one-way ANOVA, and significant differences between groups were explored by post hoc *t*-tests using the Bonferroni correction test.

TABLE 1

COMPARISON OF BEHAVIORAL MEASURES OF LEARNED HELPLESSNESS (MEANS ± SD)

	Control (n = 9)	NLH (n = 6)	LH (n = 9)
Escape latency	10.11 ± 2.37	9.83 ± 2.40	27.11 ± 6.19*
Escape failures	1.22 ± 1.30	1.50 ± 2.07	16.44 ± 6.17*

*$p < 0.001$ compared to control and nonlearned helplessness (NLH) groups; Bonferroni correction used for multiple comparisons. LH, learned helplessness.

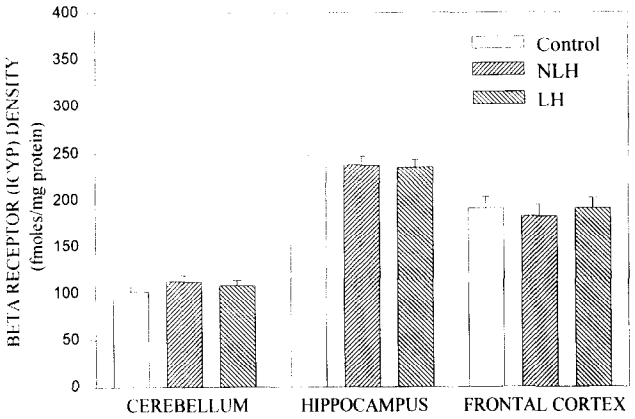

FIG. 1. Comparison of β-adrenergic binding in the cerebellum, hippocampus, and frontal cortex of control, nonlearned helpless (NLH), and learned helpless (LH) rats. Values represent means ± SE.

RESULTS

For the behavioral data, there were no significant differences among the three experimental groups in escape latencies for the five FR1 trials, showing comparable motor function. For the 25 FR2 trials, the mean escape latencies recorded from shuttle-box testing for the control group closely paralleled those of the NLH group, but were significantly lower than those of the LH group (Table 1). Similarly, the recorded escape failures for the control group and the NLH group were essentially the same, but significantly less than the LH group.

The β-receptor binding data showed no significant difference in B_{max} between any of the groups in any of the brain regions tested (Fig. 1). There were no significant differences in K_d between the groups in any of the regions (data not shown). No significant correlations between escape latencies or escape failures and the B_{max} for β-receptor binding were found in any of the tested regions (data not shown).

DISCUSSION

Behaviorally, shuttle-box testing clearly delineated the LH group from the NLH and control groups. Mean latencies and escape failures obtained in the present work were comparable to those previously reported by our laboratory (21,23,24) and by other investigators (5,13,14).

Despite the robust differences in behavior demonstrated in the present work, we found no differences in β-adrenergic receptor B_{max} or K_d between experimental groups. Thus, we failed to prove the hypothesis tested. A power analysis of our hippocampal data indicated that we could have detected 15% differences in binding density between groups (a = 0.05, b = 0.20). Furthermore, the assays in our laboratory are sensitive enough to detect a 15% decrease (p = 0.03) in cortical β-adrenergic receptor binding induced by electroconvulsive shock (1).

Our finding was somewhat surprising in light of the changes in hippocampal β-receptor binding in LH reported by other laboratories (8,17), and of the involvement of hippo-

campal (24,27) and locus coeruleus norepinephrine (30) in stress-induced depression. However, it should be noted that the relationship between β-receptors and depression, both in animal models and in humans, is not always consistent. Low densities of β-receptors have been reported in suicide victims (4). Studies of β-receptor agonists and antagonists on LH behavior (2,17) and stress-induced monoamine receptor changes in similar animal models (18) have been done, with mixed results. Also, numerous other variables besides helplessness or depression impact on adrenergic β-receptor density, and other receptor systems besides the adrenergic one are involved in depression and LH (22).

Martin et al. (16) measured β-adrenergic binding using three different ligands (including both [^{125}I]-ICYP and [^{3}H]-dihydroalprenolol ([^{3}H]-DHA)) in response deficient (RD), nondeficient (ND), and naive control Sprague–Dawley rats in a foot-shock stress–bar-press test paradigm. They found no significant differences in [^{3}H]-DHA binding density in the cortex between any of the groups. In the hippocampus, however, they reported significant increases in [^{3}H]-DHA binding density in RD compared to ND rats (23%) and to naive control rats (38%). In addition, hippocampus [^{125}I]-ICYP binding density in RD rats was significantly increased compared to ND rats (38%) and naive control rats (20%). These results implied that upregulation of β-adrenergic receptors in the hippocampus of RD rats (which correspond to our LH rats) was functionally related to the phenomenon of LH.

In comparing our results with those of Martin et al. (16), two important differences between experiments are apparent. First, a different strain of rat was used by Martin et al. (16). There is evidence that LH has a genetic component, as demonstrated by differences in susceptibility to induction of LH measured in different rat strains (31). However, in both our work and that of Martin et al. (16), the comparison was made between helpless and nonhelpless groups of rats, and strain susceptibility to LH should not have been a factor, as comparable proportions of rats developed helplessness after stress in both experiments. Also, in the present report, we did not use a naive, nontested control. However, this does not alter the finding, because naive controls were no different from ND rats in Martin et al. (16), either behaviorally or biochemically.

Second, and more important, were the procedures used to administer inescapable stress and test for behavioral depression after stress. First, the induction and testing for LH in our experiments were done with tail-shock stress and an FR2 shuttle-box test. Martin et al. (16) used the procedure of Sherman and Petty (26), which employs foot-shock stress and an FRI bar-press test. Total stress exposure was briefer in the procedure of Martin et al. (16), and 15 trials were used for testing. The effect of intensity or duration of stress on β-adrenergic receptors has been reported by Nomura et al. (19). Briefly, Wistar rats were subjected to acute (one time only) or chronic (daily for 5 days) inescapable shock. The acute inescapable shock group showed a 6% decrease in specific binding of [^{3}H]-DHA in the cortex and an 11% decrease in the hippocampus compared to controls. The chronic inescapable shock group showed a significant downregulation of [^{3}H]-DHA binding density in the cortex (15%) and a nonsignificant decrease in the hippocampus (13%) compared to controls. However, the study of Nomura et al. (19) did not include behavioral measures of the rats, making comparison with our work problematic.

Both the tail-shock or foot-shock procedures were well replicated and well established, and have been in use by the two laboratories involved for a number of years. Both procedures reliably differentiate behaviorally between helpless and nonhelpless rats, and both procedures show consistent and replicated differences between groups on several neurochemical parameters. Therefore, the most likely explanation for the difference in findings between our work and that of Martin et al. (16) is the difference in LH training and testing procedures, which apparently affect noradrenergic hippocampal receptors differently.

In summary, our findings do not replicate either the stress-induced decrease in β-adrenergic receptor binding reported by Nomura et al. (19) or the LH associated increase in hippocampus β-adrenergic receptor binding reported by Martin et al. (16). In this regard, it is interesting to review a recent report on β-adrenergic receptor binding, in another animal model of depression, olfactory bulbectomy. Dennis et al. (3) failed to replicate an earlier study (29) reporting a 30% increase in affinity with no change in B_{max}, and also failed to replicate another study (10) reporting an increase in B_{max} in hippocampus. Therefore, discrepant results in adrenergic receptor binding studies has also been found in another animal model of depression.

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