Pharmacology Biochemistry and Behavior, Vol. 45, pp. 1-7, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

β-Adrenergic Receptors are Involved in Stress-Related Behavioral Changes

A. LAUREL GORMAN AND ADRIAN J. DUNN[1]

Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, P.O. Box 33932, Shreveport, LA 71130-3932

GORMAN, A. L. AND A. J. DUNN. *β-Adrenergic receptors are involved in stress-related behavioral changes.* PHARMACOL BIOCHEM BEHAV **45**(1) 1-7, 1993.—Cerebral noradrenergic systems have been implicated in stress-related changes in behavior. Previous studies with receptor antagonists suggested that α_1-adrenergic receptors were involved in defensive withdrawal in rats and in investigatory behavior in mice tested in the multicompartment chamber. However, β-adrenoreceptor antagonists attenuated the restraint- and ICV CRF-induced changes in defensive withdrawal, suggesting that β-adrenergic receptors may also be involved in stress-related responses. To determine whether the β-adrenergic antagonist effect was limited to rats tested in the defensive withdrawal model, we studied the effects of L-propranolol in two other behavioral models. Propranolol pretreatment (2.5 mg/kg, IP) prevented the restraint-induced changes in the behavior of mice observed in the multicompartment chamber and the elevated plus-maze. It also decreased the plasma corticosterone response measured in restrained mice after plus-maze testing. To investigate further the role of central β-adrenergic receptors in defensive withdrawal, the effects of the β-adrenoreceptor agonist isoproterenol were tested. Isoproterenol (0.3-10 μg, ICV) produced a dose-dependent increase in defensive withdrawal, statistically significant after 3 and 10 μg. Propranolol prevented the isoproterenol-induced defensive withdrawal, suggesting that the effect of isoproterenol resulted from stimulation of β-adrenergic receptors. These results support earlier data suggesting the involvement of CNS β-adrenergic receptors in stress-related behavioral changes and suggest that β-adrenergic agonists exert anxiolytic effects that differ from those of the benzodiazepines.

Elevated plus-maze Defensive withdrawal Exploratory behavior β-Adrenergic receptors Anxiety Stress Propranolol Isoproterenol

SUBSTANTIAL evidence suggests that noradrenergic systems are involved in stress-related behavioral responses (7,11,31). In rats, novelty or restraint induces defensive withdrawal, a behavioral pattern characterized by increased time spent in a small enclosed chamber relative to exploration of an open field (33,38). This change was mimicked by ICV administration of the selective α_1-adrenergic agonist phenylephrine in unrestrained animals, and the restraint-induced changes were prevented by the α_1-adrenergic antagonist prazosin (38). In mice, restraint or ICV phenylephrine decreased investigatory behavior, quantified as a reduction in the mean stimulus-contact times measured in the multicompartment chamber [MCC; (2-4)]. Prazosin prevented these restraint-induced changes (4). Thus, in both defensive withdrawal and in the MCC pharmacological analysis suggests that the restraint-induced behavioral changes were the result of α_1-adrenergic stimulation.

However, β-adrenergic antagonists have also been shown to exhibit anxiolytic properties in several behavioral models (8,34,38), suggesting that β-adrenergic receptors are also involved in anxiety-like responses. L-Propranolol (2.5 or 5 mg/kg) decreased defensive withdrawal in naive rats and prevented that induced by restraint (38). Propranolol also prevented the changes in defensive withdrawal induced by ICV corticotropin-releasing factor [CRF; (37,38)]. The changes induced by CRF were not altered by peripherally administered CGP-12177, a β-adrenergic antagonist that penetrates the brain poorly, nor by ICI 118,551, a β_2-adrenergic antagonist (37). However, the β_1-adrenergic antagonists, atenolol and CG-20712A, administered ICV attenuated the CRF-induced defensive withdrawal (37), suggesting the involvement of central β_1-receptors.

Our first objective was to extend our understanding of the involvement of β-adrenergic receptors in stress-related behavioral changes by determining whether a β-adrenergic antagonist altered the effects of restraint on mice tested in the MCC and the elevated plus-maze. The MCC was chosen because previous studies have shown that the investigatory behavior measured in this model was sensitive to restraint (1-4). The elevated plus-maze test was selected as a commonly used model for anxiety (13,22,27). Because β-adrenergic antagonists have been shown to attenuate restraint-induced changes in defensive withdrawal, our second objective was to determine whether stimulation of central β-adrenergic receptors elicited defensive withdrawal.

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

METHOD

Animals

Adult, male Sprague-Dawley rats (250–300 g) were obtained from Harlan-Sprague-Dawley, Inc. (Indianapolis, IN) and male albino VAF-plus CD-1 mice (25–32 g) were obtained from Charles River (Raleigh-Durham, NC). Rats and mice were housed individually in plastic cages placed in a light- and temperature-controlled room (lights on from 7:00 a.m.–7:00 p.m.) with free access to food and water.

Cannulation and Injection of Rats

A 9-mm stainless steel 23-ga guide cannula was implanted above the lateral ventricle on each side of the brain as previously described (38) according to the following stereotaxic coordinates: A-P, 0.4 mm (from bregma); L, 2.0 mm; and D, 4.0 mm below the skull surface. The surgery was performed under phenobarbital anesthesia (55 mg/kg, IP) given 10 min after atropine (1.0 mg/kg, IP). On the day of the experiment, the stylet was removed and a 9-mm 31-ga injection needle inserted into the guide cannula. The needle was attached to a 10-μl Hamilton syringe (Hamilton, Reno, NV) by approximately 30 cm of polyethylene tubing (Clay Adams PE 10), and 2 μl of sterile filtered artificial cerebrospinal fluid (aCSF) or sterile filtered isoproterenol (ISO) was injected into each cannula slowly over a 1-min period. At the completion of all experiments, rats were sacrificed and the cannula placements verified by the injection of 1 μl methylene blue dye into each cannula.

Drugs

(-)Isoproterenol (bitartrate salt) and L-propranolol HCl were obtained from Sigma Chemical Co. (St. Louis, MO). Both solutions were prepared fresh on the day of the experiment. Propranolol was dissolved in sterile filtered saline at a concentration of 0.25 mg/ml (mice) or 2.5 mg/ml (rats), and isoproterenol was dissolved in aCSF.

Behavioral Procedures

MCC. We used the behavioral apparatus of Arnsten et al. (1), modified for use in mice as previously described (2–4). The apparatus consisted of a Plexiglas box with nine interconnecting compartments with a 1-in. diameter hole in the center of each compartment. A 3.0-cm sphere wire mesh stimulus was mounted below each hole, and a stimulus contact was defined when the mouse made contact with this wire. Each mouse was placed in the center compartment of the MCC, and various behaviors were scored for 25 min using an NEC8201A computer modified for use as an event recorder (Stoelting, Chicago, IL, Model #47250X). The observer was blind to the treatment group, and no animal was exposed to the apparatus more than once. The behaviors measured were the number of crosses between compartments, rears, grooming, and the number of stimulus contacts and the stimulus-contact time. Following each test, the apparatus was cleaned with 0.1% acetic acid.

Elevated Plus-Maze. This test adapted for use in mice was used as described by Lister (19). It consisted of four perpendicular arms made of black Plexiglas elevated 38.5 cm above the floor. The arms extended from the central platform (5 × 5 cm). Two arms were open (30 × 5 cm) and two arms were enclosed by transparent walls (30 × 5 × 15 cm). Illumination of the apparatus was by a 25-W soft white light placed directly above the center, and testing was conducted in a room separate from the housing and surgical facilities between 9:00 a.m. and 2:00 p.m. To initiate a test, the mouse was placed in the center facing a closed arm and scored for 5 min by an observer blind to the treatment groups. Each animal was unfamiliar with the apparatus and testing room, and no animal was tested more than once. Following each test, the apparatus was cleaned with 0.1% acetic acid. The following behaviors were scored on an NEC computer: the amount of time spent in the open and closed arms; the total number of entries into the open and closed arms; the total number of rears and the amount of time spent rearing.

Restraint. Mice to be restrained were transported to a room separate from the housing room and the behavioral testing room. Each mouse was placed in a 50-ml enclosed, darkened plastic centrifuge tube with air holes at the tip, and tape was applied to the open end so that the animal could not escape. Mice were restrained for 40 min.

Defensive withdrawal. The task was used as described by Takahashi et al. (33) and previously reported (6,38,39). The apparatus consisted of a white opaque Plexiglas open field (100 × 110 × 35 cm) with the floor marked in 20 × 20-cm squares. A cylindrical, galvanized chamber, 15 cm deep and 13 cm in diameter, with one open end, was secured to the floor and wall 40 cm away from a corner. Illumination of the apparatus was by a 22-W fluorescent light placed above the center of the field. Defensive withdrawal testing was conducted in a room separate from the housing and surgery facilities. A white noise generator was used to obscure background noise. To initiate a session, the rat was placed in the chamber and its behavior was recorded for 15 min by a videocamera mounted on the wall above the apparatus. During the behavioral recording session, the rat was alone in the room. After each test, the apparatus was cleaned with 0.1% acetic acid. The behavioral parameters measured were defined as follows: latency, the time from the beginning of the experiment until the rat placed all four paws into the open field; total time in the chamber (TTIC), the total time the animal spent in the chamber during the experimental session; mean time in the chamber (MTIC), the TTIC divided by the total number of reentries plus one (initial placement of the rat into the chamber); locomotor velocity (LV), the total number of lines crossed divided by the time spent outside of the chamber; rear frequency (RF), the total number of rears divided by the time spent outside of the chamber.

Rats were familiarized with the apparatus by being placed in the center of the open field for 10 min on the day before the experiment. The chamber was removed during the familiarization process to facilitate exploration of the open field. Each rat was placed in the chamber for 1 min after the 10-min period so that it would also be familiar with the chamber. Animals were tested in a repeated-measures design such that each rat received every combination of treatments. No animal was tested on isoproterenol or propranolol on 2 consecutive days. For this reason, animals were tested more than once on aCSF, saline/aCSF, and propranolol/aCSF. For each of these treatment groups, the mean of the rat's scores on each behavioral parameter was used for statistical analysis. No animal was tested for more than 4 days in any week.

Corticosterone Assay

Each mouse was transported to a separate room immediately after completion of the elevated plus-maze test. Trunk blood was collected into heparinized Eppendorf tubes and the

plasma was separated. Plasma corticosterone was assayed by radioimmunoassay performed according to the method of Gwosdow-Cohen et al. (18) after extraction from 5-μl samples of plasma using methylene chloride.

Statistical Analysis

For the MCC and elevated plus-maze experiments, the data were analyzed by analysis of variance (ANOVA) with Duncan's test using SuperANOVA (Macintosh). The dose-response data for isoproterenol were analyzed by Dunnett's *t*-test, and the ability of propranolol to block isoproterenol-induced defensive withdrawal was analyzed by ANOVA with Duncan's test posthoc. A logarithmic transformation of the defensive withdrawal measures was used to normalize the data for statistical analysis.

RESULTS

Multicompartment Chamber Behavior

To determine whether β-adrenoreceptors are involved in behavioral changes observed in the MCC, mice were pretreated with saline or propranolol prior to being restrained and tested in the MCC. As in previous experiments (2–4), the only statistically significant behavioral change induced by restraint was a reduction in the mean stimulus-contact time (MSCT) compared with the quiet controls (Fig. 1A). Pretreatment with L-propranolol (2.5 mg/kg) prevented this restraint-induced change but did not alter the behaviors scored in unrestrained animals. A 1.0-mg/kg dose of propranolol did not significantly alter the effects of restraint (data not shown). Neither restraint nor propranolol altered the number of compartment entries (Fig. 1B), indicating that mice were not sedated by either treatment.

Elevated Plus-Maze Behavior in Mice

To determine whether β-adrenoreceptors are involved in behavioral changes observed in the elevated plus-maze, mice were pretreated with saline or propranolol prior to being restrained and tested. Restraint significantly reduced the percentage of entries made into the open arms when compared with quiet controls (Fig. 2A). Restraint tended to decrease the percentage of time spent in the open arms (Fig. 2B) but this change was not statistically significant. Pretreatment with propranolol (2.5 mg/kg) prevented these restraint-induced effects. No statistically significant changes were observed in the total number of entries onto the arms (SAL-Q 23.6 ± 6.6; SAL-R 20.0 ± 4.9; PRO-Q 25.7 ± 8.1; PRO-R 32.0 ± 5.7). In pilot studies, a 1.0-mg/kg dose of propranolol did not significantly alter the restraint-induced changes in behavior, whereas a 10-mg/kg dose was sedative.

Blood samples were collected immediately after the behavioral test for assay of plasma corticosterone. Concentrations of plasma corticosterone were increased in restrained mice compared with unrestrained controls (Fig. 3). Pretreatment with propranolol attenuated the restraint-related increase but did not alter plasma corticosterone in unrestrained mice. Both the effects of restraint in saline-treated mice and of propranolol in restrained mice were statistically significant.

Effects of Isoproterenol on Defensive Withdrawal Behavior in Rats

To determine whether stimulation of cerebral β-receptors is involved in stress-related behavior, we tested rats in defensive withdrawal after ICV administration of isoproterenol. In three separate experiments, ICV isoproterenol (in the range 0.1–10 μg) produced dose-dependent increases in defensive withdrawal: increases in the latency to emerge from the small chamber and in the mean and total times spent in the chamber (MTIC and TTIC). Although the absolute values for the latency and time in the chamber varied between experiments, they were consistently statistically significant at doses of 3 μg or more. Therefore, we tested the specificity of the effect only at the 3- and 10-μg doses. Figure 4 shows that both 3 and 10 μg of isoproterenol increased the latency to emerge from the small chamber, as well as the MTIC and TTIC. Isoproterenol

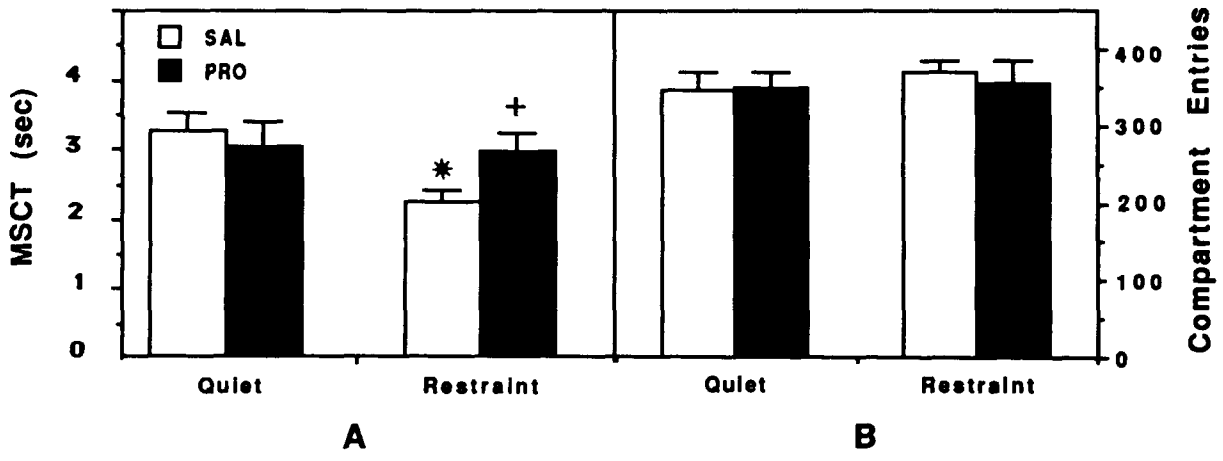

FIG. 1. Effects of restraint with and without propranolol pretreatment on the behavior of mice tested in the MCC. Mice were injected IP with 2.5 mg/kg propranolol (PRO) or the same volume of saline (SAL) 30 min prior to being restrained (R) for 40 min or returned to their home cages as quiet controls (Q). Mice were then scored in the MCC for 25 min. For SAL/Q, $n = 11$; SAL/R, $n = 12$; PRO/Q and PRO/R, $n = 8$. (A) Mean stimulus-contact time (MSCT: mean ± SEM). ANOVA indicated a statistically significant interaction between PRO and R, $F(1, 5) = 76.9$, $p < 0.001$, and the effects of restraint in saline-treated mice and of propranolol in restrained mice were significant by Duncan's test ($p < 0.01$). (B) Compartment entries. ANOVA did not indicate any statistically significant effects of the treatments. $^{*}p < 0.05$ compared with SAL/Q, $^{+}p < 0.05$ compared with SAL/R.

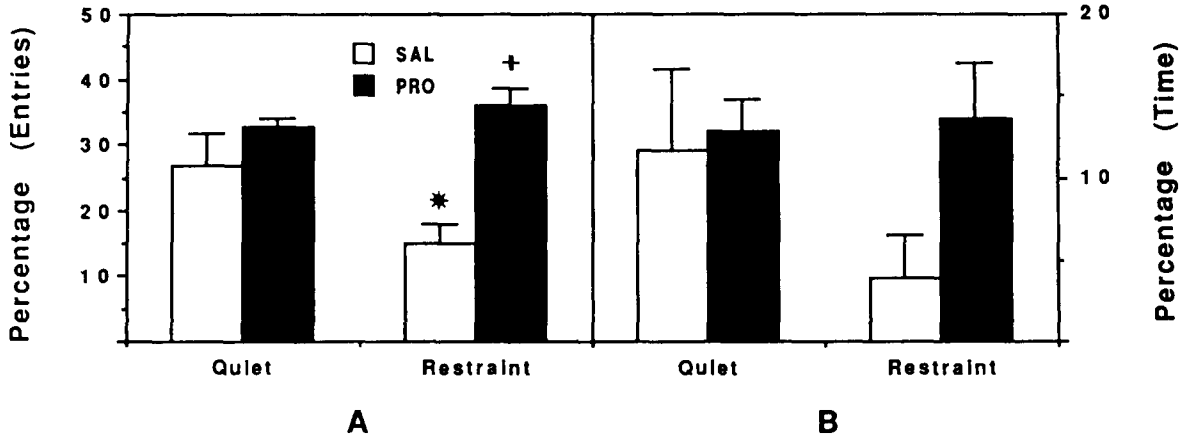

FIG. 2. Effects of restraint with and without propranolol pretreatment on the behavior of mice tested in the elevated plus-maze. Naive mice were injected with propranolol (PRO, 2.5 mg/kg, IP) or the same volume of saline (SAL) 30 min prior to being restrained (R) or returned to their home cages as quiet controls (Q) for 40 min. Immediately after this, mice were tested in the plus-maze for 5 min. $n = 6$, except for SAL/Q, in which $n = 5$. (A) Mean ± SEM percentage of entries made into the open arms. ANOVA indicated a significant interaction between PRO and restraint, $F(1, 19) = 6.05$, $p < 0.05$, and Duncan's test indicated significant effects of restraint in saline-treated mice and of propranolol in restrained mice ($p < 0.05$). (B) Mean percentage of time spent on the open arms. ANOVA did not indicate any statistically significant effects of the treatments. $^{*}p < 0.05$ compared with SAL/Q, $^{+}p < 0.05$ compared with SAL/R.

did not significantly alter the line crossings or rears (data not shown). To test whether the effect of isoproterenol was specific for β-adrenergic receptors, mice were pretreated with propranolol. A dose of 2.5 mg/kg propranolol was used because it had attenuated restraint- and CRF-induced defensive withdrawal in previous studies (39) without producing apparent sedative effects. The propranolol treatment essentially prevented each of the isoproterenol-induced changes (Fig. 4).

DISCUSSION

In previous studies of mouse behavior in the MCC, the stimulus-contact times were selectively reduced by various stressors, including restraint (1–4). In the present study, pro-

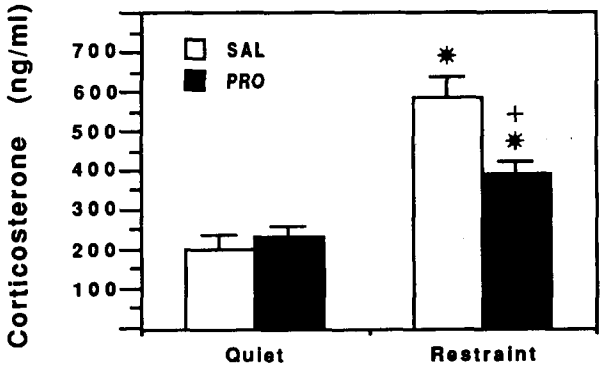

FIG. 3. Mean plasma corticosterone concentrations measured in trunk blood collected immediately after plus-maze testing. The same experiment as Fig. 2 (1 PRO/R blood sample was lost). ANOVA indicated a significant interaction between PRO and restraint, $F(1, 18) = 7.0$, $p < 0.05$, and Duncan's test indicated that the effects of restraint in saline-treated mice and of propranolol in restrained mice were statistically significant. $^{*}p < 0.05$ compared with SAL/Q, $^{+}p < 0.05$ compared with SAL/R.

attenuated the restraint-induced decrease in the mean stimulus-contact time without altering the number of compartment entries, suggesting that the drug treatment did not cause sedation. In the elevated plus-maze, restraint decreased the proportion of open arm entries, suggesting an anxiogenic effect. Propranolol prevented this restraint-induced change but did not significantly alter the behavior of unrestrained animals. Restraint pretreatment caused increased plasma concentrations of corticosterone relative to those of unrestrained mice tested in the elevated plus-maze. This response to restraint was attenuated by propranolol pretreatment. This finding suggests that propranolol reduced the aversive nature of the exposure to the apparatus because there is no evidence that propranolol acts directly to alter corticotropin (ACTH) or corticosterone secretion (29). Thus, the results of these studies extend our observations on the "antistress" or "anxiolytic" effects of propranolol in defensive withdrawal in rats (38) to another species and two different behavioral tasks.

Propranolol readily crosses the blood-brain barrier (20), and its effects on restraint-induced behavior may be centrally and/or peripherally mediated. Because previous studies in rats have suggested that a central mechanism is involved in the anxiolytic properties of β-adrenergic receptor antagonists (12,37), we tested the effects of a β-adrenergic agonist administered ICV on defensive withdrawal. Rats given isoproterenol showed a preference for the small enclosed chamber over the open field. Isoproterenol did not alter the rearing frequency or the number of lines crossed when animals were in the open field, indicating that stimulation of central β-adrenergic receptors induces defensive withdrawal without altering locomotor activity. Pretreatment with 2.5 mg/kg propranolol prevented the defensive withdrawal behavior induced by isoproterenol, suggesting that the effects of isoproterenol were mediated by activation of β-adrenergic receptors. Propranolol has affinity for both 5-HT_{1A} and 5-HT_{1B} receptors (25), and it has been suggested that some of its anxiolytic properties could be related to its effects on serotonergic systems (16). Our results

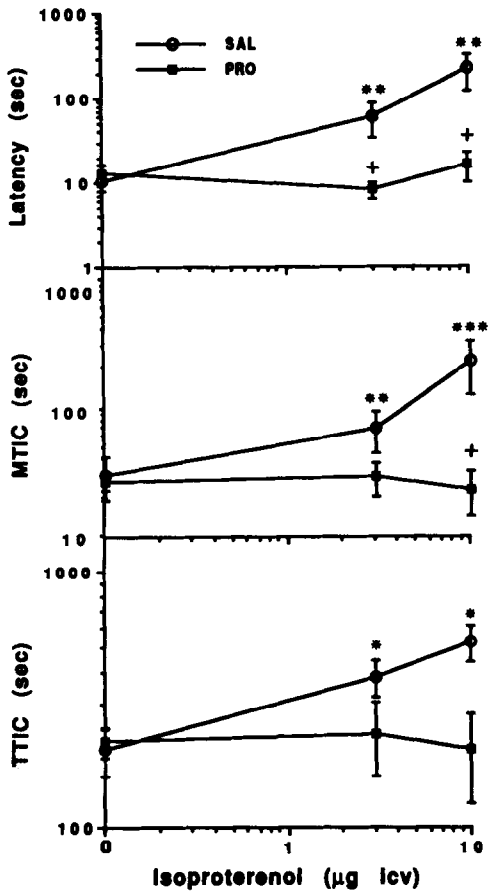

FIG. 4. Effects of ICV isoproterenol and propranolol on defensive withdrawal in rats. Rats were familiarized with the open field and then tested on subsequent days. Propranolol (PRO; 2.5 mg/kg, IP) or SAL was injected 20 min prior to injecting either aCSF or isoproterenol (ISO; 3 or 10 μg ICV), and behavioral testing commenced 20 min after the second injection. All rats (n = 10) received all combinations of drugs, but no animal received PRO or ISO on 2 consecutive days. (A) Latency. (B) MTIC. (C) TTIC. For the latency, ANOVA indicated a significant interaction between PRO and 3 μg ISO, $F(1, 59) = 17.9$, $p < 0.01$, and between PRO and 10 μg ISO, $F(1, 59) = 11.7$, $p < 0.01$. Dunnett's t-test indicated significant differences between 3 μg ISO and aCSF, $t(3, 27) = 3.50$, $p < 0.01$, and between 10 μg ISO and aCSF, $t(3, 27) = 5.5$, $p < 0.01$. The effects of PRO in 3- and 10-μg ISO-treated rat were statistically significant by Duncan's test. For the MTIC, ANOVA indicated a significant interaction between PRO and 3 μg ISO, $F(1, 59) = 5.9$, $p < 0.05$, and between PRO and 10 μg ISO, $F(1, 59) = 16.3$, $p < 0.01$. Dunnett's t-test indicated significant differences between 3 μg ISO and aCSF, $t(3, 27) = 4.4$, $p < 0.01$, and between 10 μg ISO and aCSF, $t(3, 27) = 10.3$, $p < 0.001$. The effect of PRO in 10-μg ISO-treated rats was statistically significant by Duncan's test. For the TTIC, ANOVA indicated a significant interaction between PRO and 3 μg ISO, $F(1, 59) = 5.9$, $p < 0.05$, and between PRO and 10 μg ISO, $F(1, 59) = 8.8$, $p < 0.05$. Dunnett's t-test indicated significant differences between 3 μg ISO and aCSF, $t(3, 27) = 2.9$, $p < 0.05$, and between 10 μg ISO and aCSF, $t(3, 27) = 2.7$, $p < 0.05$. $^{*}p < 0.05$; $^{**}p < 0.01$ compared to aCSF/SAL, $^{+}p < 0.05$ compared with SAL/ISO.

with isoproterenol suggest that defensive withdrawal can be by direct activation of central β-adrenergic receptors and provide strong support for the conclusion that noradrenergic systems mediate these behavioral responses.

Cerebral CRF has also been implicated in stress-related behaviors (10,26). ICV administration of CRF has been shown to mimic the novelty- or restraint-induced changes in defensive withdrawal (6,33,37,38), in the MCC (2–4), and in the elevated plus-maze (14), and the CRF antagonist α-helical CRF9-41 (αhCRF) has been shown to reverse the effects of novelty or restraint (3,5,33,38). Thus, interactions between noradrenergic systems and CRF-containing neurons in the brain may be important in the modulation of stress-related behavioral changes.

Previous behavioral pharmacological studies have focused on the involvement of α_1-adrenergic receptors because treatment with the α_1-adrenergic antagonist, prazosin, prevents the effects of restraint on defensive withdrawal and in the MCC, and ICV administration of the selective α_1-adrenergic agonist, phenylephrine, mimics those effects (4,38). In both behavioral models, αhCRF prevented the effects of phenylephrine but prazosin did not attenuate the CRF-induced behavioral changes (4,38). This suggests that α_1-receptors stimulate (directly or indirectly) CRF neurons involved in the behavioral responses. Exposure to restraint or an unfamiliar environment may activate noradrenergic systems, which in turn stimulate CRF release via an α_1-adrenergic mechanism. This neuronal arrangement is consistent with data on the regulation of CRF release by hypophysiotropic neurons, suggesting that α_1-adrenergic receptors stimulate CRF-containing neurons in the paraventricular nucleus (29,32).

However, most noradrenergic terminals are not associated with CRF-containing neurons. Moreover, the role of β-adrenergic receptors is ignored in the above model. As discussed above, propranolol can prevent the behavioral responses to restraint in defensive withdrawal (38), in the MCC (Fig. 1), and in the elevated plus-maze (Fig. 2). Further, β-adrenergic antagonists can prevent the CRF-induced increases in defensive withdrawal (37,38) and in conditioned fear (8). By contrast, α_1-adrenergic antagonists did not prevent the CRF-induced behavioral changes in the MCC (4) or in defensive withdrawal (38). Together, these results suggest that α_1-adrenergic receptors are involved upstream of CRF neurons and β-adrenergic receptors are involved downstream. The latter arrangement is consistent with electrophysiological data indicating that ICV administration of CRF increases the firing rate of locus coeruleus noradrenergic neurons (36) and neurochemical data indicating that ICV administration of CRF increases concentrations of norepinephrine (NE) catabolites in several brain regions (6,9,24) and extracellular concentrations of NE assessed by in vivo microdialysis (21). It is likely that CRF-containing neurons interact with noradrenergic neurons in different ways in different anatomic locations. Thus resolution of these apparently conflicting data may be obtained from a more detailed neuroanatomic analysis of the neuronal substrates involved in these stress-related behaviors.

Antianxiety effects of β-adrenergic antagonists have been reported in a limited number of behavioral studies, suggesting that β-adrenergic involvement may be limited to certain types of stress-related behaviors. Pellow et al. (28) reported that propranolol, unlike the prototypical anxiolytics, failed to produce alterations in open-arm activity in the plus-maze in rats. Because benzodiazepine treatments produce marked effects in this model (14,22), they concluded that the β-adrenergic antagonists lacked efficacy as anxiolytics in this task. Our results also indicate that propranolol did not alter open-arm entries or mean stimulus-contact times in unrestrained mice but did attenuate both the behavioral and plasma corticosterone changes induced by restraint. It appears then that propranolol does not affect behavioral responses to novelty but appears rather to be anxiolytic when a stronger stressor such

as restraint is used. Thus, β-adrenergic antagonists may have an anxiolytic profile different from the benzodiazepines. We reported earlier that chlordiazepoxide attenuated novelty- and restraint-induced defensive withdrawal (38), but we have not observed clear-cut effects of benzodiazepines in the MCC.

Conflicting results in the literature support the notion that the β-adrenergic antagonists exert anxiolytic effects different from the benzodiazepines. Clinically, propranolol has been used to treat performance anxiety (19), but its efficacy has been attributed to its ability to relieve peripheral autonomic symptoms in anxious patients (17,23,35). The β-adrenergic antagonists have been most effective in situations where the anxious patient tended to have many autonomic symptoms and was unable to identify a specific, fear-evoking stimulus (35). In a conflict test, Fontana et al. (15) found that propranolol did not increase punished drinking suppressed by different shock intensities. However, Terry and Salmon (34) showed that the ability of β-adrenergic antagonists to increase punished responding was dependent upon stimulus salience and the type of discriminative stimulus used. In their study, a limited range of doses of propranolol increased responding suppressed by low-intensity shock when a flashing light was used to signal the punishment schedule. They speculated that the flashing lights increased the arousal level of animals, and their data suggest β-adrenergic mediation of this effect. By contrast, chlordiazepoxide increased punished responding at higher levels of response suppression (30) independent of the discriminative stimulus used (34).

In conclusion, our results indicate that β-adrenergic receptors are involved in stress-related behaviors in both rats and mice. Consistent with our earlier study in rats, propranolol attenuated restraint-induced behavioral and endocrine changes in mice. We also provided evidence that stimulation of central β-adrenergic receptors with isoproterenol mimics a stress-related behavioral response and that this response was prevented by pretreatment with propranolol, suggesting that the effect was mediated by β-adrenergic receptors.

ACKNOWLEDGEMENTS

This research was supported by Grant NS27283 from the NIH. The authors thank Yvette Chapman and Michael Antoon for behavioral scoring in the MCC and Dr. David Saphier and Dr. Jim O'Donnell for their helpful suggestions.

REFERENCES

1. Arnsten, A. F. T.; Berridge, C. W.; Segal, D. S. Stress produces opioid-like effects on investigatory behavior. Pharmacol. Biochem. Behav. 22:803–809; 1985.
2. Berridge, C. W.; Dunn, A. J. Corticotropin-releasing factor elicits naloxone-sensitive stress-like alterations in exploratory behavior in mice. Regul. Peptides 16:83–93; 1986.
3. Berridge, C. W.; Dunn, A. J. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. Horm. Behav. 21:393–401; 1987.
4. Berridge, C. W.; Dunn, A. J. Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF. J. Neurosci. 9:3513–3521; 1989.
5. Britton, K.; Merlo Pich, E.; Heinrichs, S. C.; Miczek, K. A.; Rivier, C.; Koob, G. F. Behavioral and endocrine responses to social conflict stress in rats: Effects of a CRF antagonist, α-helical CRF9-41. Soc. Neurosci. Abstr. 17:1417; 1991.
6. Butler, P. D.; Weiss, J. M.; Stout, J. C.; Nemeroff, C. B. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. J. Neurosci. 10:176–183; 1990.
7. Charney, D. S.; Heninger, G. R.; Breier, A. Noradrenergic function in panic anxiety: Effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. Arch. Gen. Psychiatry 41:751–763; 1984.
8. Cole, B. J.; Koob, G. F. Propranolol antagonizes the enhanced conditioned fear produced by corticotropin-releasing factor. J. Pharmacol. Exp. Ther. 247:902–910; 1987.
9. Dunn, A. J.; Berridge, C. W. Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems. Pharmacol. Biochem. Behav. 27:685–691; 1987.
10. Dunn, A. J.; Berridge, C. W. Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? Brain Res. Rev. 15:71–100; 1990.
11. Dunn, A. J.; Kramarcy, N. R. Neurochemical responses in stress: Relationships between HPA and catecholamine systems. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H. eds. Handbook of psychopharmacology. vol. 18. New York: Plenum Press; 1984:455–515.
12. Durel, L. A.; Krantz, D. S.; Barrett, J. E. The antianxiety effect of beta-blockers on punished responding. Pharmacol. Biochem. Behav. 25:371–374; 1986.
13. File, S. E. Interactions of anxiolytic and antidepressant drugs with hormones of the hypothalamic–pituitary–adrenal axis. Pharmacol. Ther. 46:357–375; 1990.
14. File, S. E.; Johnston, A. L.; Baldwin, H. A. Anxiolytic and anxiogenic drugs: Changes in behaviour and endocrine responses. Stress Med. 4:221–230; 1988.
15. Fontana, D. J.; McCloskey, T. C.; Jolly, S. K.; Commissaris, R. L. The effects of betaantagonists and anxiolytics on conflict behavior in the rat. Pharmacol. Biochem. Behav. 32:807–813; 1989.
16. Graeff, F. G.; Audi, E. A.; Almeida, S. S.; Graeff, E. O.; Hunziker, M. H. Behavioral effects of 5-HT receptor ligands in the aversive brain stimulation, elevated plus-maze, and learned helplessness tests. Neurosci. Biobehav. Rev. 14:501–506; 1990.
17. Granville-Grossman, K. L.; Turner, P. The effect of propranolol on anxiety. Lancet i:788–790; 1966.
18. Gwosdow-Cohen, A.; Chen, C. L.; Besch, E. L. Radioimmunoassay (RIA) of serum corticosterone in rats. Proc. Soc. Exp. Biol. Med. 170:29–34; 1984.
19. James, I.; Burhoyne, W.; Greenwood, D. Effects of ICI118,551, a beta$_2$adrenoceptor antagonist on anxiety associated with public speaking. Collegium Internationale Neuropsychopharmacologium (CINP), Abstract 141, 1986.
20. Johnsson, G.; Regardh, C.-G. Clinical pharmacokinetics of β-adrenoceptor blocking drugs. Clin. Pharmacokinet. 1:233–263; 1976.
21. Lavicky, J.; Dunn, A. J. Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. J. Neurochem. 60; 1993.
22. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl.) 92:180–185; 1987.
23. Liu, H. H.; Milgrom, P.; Fiset, L. Effect of a beta-adrenergic blocking agent on dental anxiety. J. Dent. Res. 70:1306–1308; 1991.
24. Matsuzaki, I.; Takamatsu, Y.; Moroji, T. The effects of intracerebroventricularly injected corticotropin-releasing factor (CRF) on the central nervous system: Behavioural and biochemical studies. Neuropeptides 13:147–155; 1989.
25. Middlemiss, D. N. Stereoselective blockade of [^{3}H]5HT binding sites and at the 5HT autoreceptor by propranolol. Eur. J. Pharmacol. 101:289–293; 1984.
26. Owens, M. J.; Nemeroff, C. B. Physiology and pharmacology

of corticotropin-releasing factor. Pharmacol. Rev. 43:425–471; 1991.
27. Pellow, S.; File, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529; 1985.
28. Pellow, S.; Johnston, A. L.; File, S.E. Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG 7142 using the elevated plus-maze test in the rat. J. Pharm. Pharmacol. 39:917–928; 1987.
29. Plotsky, P. M.; Cunningham, E. T.; Widmaier, E. P. Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. Endocrine Rev. 10:437–458; 1989.
30. Rawlins, J. N. P.; Feldon, J.; Salmon, P.; Gray, J. A.; Garrud, P. The effects of chlordiazepoxide HCl administration upon punishment and conditioned suppression in the rat. Psychopharmacology (Berl.) 70:317–322; 1980.
31. Redmond, D. E.; Huang, Y. H. New evidence for a locus coeruleus–norepinephrine connection with anxiety. Life Sci. 25:2149–2162; 1979.
32. Saphier, D.; Feldman, S. Adrenoceptor specificity in the central regulation of adrenocortical secretion. Neuropharmacology 28: 1231–1237; 1989.
33. Takahashi, L. K.; Kalin, N. H.; Vanden Burgt, J. A.; Sherman, J. E. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. Behav. Neurosci. 103: 648–654; 1989.
34. Terry, P.; Salmon, P. Anxiolytic-like action of beta-blockers: Effects of stimulus salience. Pharmacol. Biochem. Behav. 39: 597–603; 1991.
35. Tyrer, P. Anxiolytics not acting at the benzodiazepine receptor: Beta blockers. Prog. Neuropsychopharmacol. Biol. Psychiatry 16:17–26; 1992.
36. Valentino, R. J.; Foote, S. L.; Aston-Jones, G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Res. 270:363–367; 1983.
37. Yang, X.-M.; Dunn, A. J. Central β_1-adrenergic receptors are involved in CRF-induced defensive withdrawal. Pharmacol. Biochem. Behav. 36:847–851; 1990.
38. Yang, X.-M.; Gorman, A. L.; Dunn, A. J. The involvement of central noradrenergic systems and corticotropin-releasing factor in defensive withdrawal behavior in rats. J. Pharmacol. Exp. Ther. 255:1064–1070; 1990.