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Corticotropin-Releasing Factor and Defensive Withdrawal: Inhibition of Monoamine Oxidase Prevents Habituation to Chronic Stress

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WARD, H. E., E. A. JOHNSON, I. J. GOODMAN, D. L. BIRKLE, D. J. COTTRELL AND A. J. AZZARO. *Corticotropin-releasing factor and defensive withdrawal: Inhibition of monoamine oxidase prevents habituation to chronic stress*. PHARMACOL BIOCHEM BEHAV **60**(1) 209–215, 1998.—There is growing evidence for a role of extrahypothalamic corticotropin-releasing factor (CRF) in the pathogenesis of anxiety. A modified form of the defensive withdrawal test was used to test the anxiogenic effects of acute administration of intracerebroventricular (1 μg, ICV) CRF in adult male rats. Habituation to the mild stress of daily handling and subcutaneous (SC) saline injection over 2–6 weeks abolished the anxiogenic effects of exogenous CRF. At 6 weeks this habituation also resulted in attenuation of baseline withdrawal behavior. CRF receptor binding was significantly decreased in the amygdala of chronically handled animals and may have been responsible for this habituation phenomenon. Comparison of rats treated with the monoamine oxidase (MAO) inhibitor, phenelzine [3 mg/kg, SC, daily for 2–6 weeks] to the saline-treated groups revealed a failure to habituate to the chronic handling, as the baseline withdrawal (after injection of artificial CSF) by the phenelzine-treated animals was not different from the baseline withdrawal by unhandled rats. In comparison to rats treated chronically with saline, phenelzine treatment enhanced the anxiogenic effect of CRF. In summary, habituation to a mild chronic stress decreased baseline defensive withdrawal. Intraventricular administration of CRF produced an anxiogenic response as measured in the defensive withdrawal test, which was lost through exposure to mild chronic stress. Two or 6 weeks of daily handling and SC saline injection caused a downregulation of CRF receptors in the amygdala, which could account for the behavioral habituation and the loss of CRF-induced defensive withdrawal. Phenelzine treatment concurrent with mild chronic stress prevented habituation and maintained the anxiogenic effect of CRF in spite of the downregulation of CRF receptors in the amygdala. © 1998 Elsevier Science Inc.

Corticotropin-releasing factor Anxiety Defensive withdrawal Phenelzine Behavior Amygdala Monoamine oxidase inhibitor Rat Chronic stress MAO Antidepressant CRF

ANXIETY disorders are common and can be debilitating. Major advances have been made over the past 10 years in the diagnosis and treatment of conditions such as panic disorder, obsessive compulsive disorder, and social phobia (12,34,45, 48,55). This has occurred in large part from the introduction of more selective psychotropic drugs into clinical practice (5,11,46,53). Much of our understanding regarding synaptic mechanisms involved in anxiety disorders has been inferred from the therapeutic effect of agents that alter brain levels of norepinephrine, serotonin, and γ-aminobutyric acid (GABA). However, there remains a paucity of information regarding the basic neurobiological processes that mediate normal anxiety and pathologic anxiety states. Although the subjective experience of anxiety can be studied only in human subjects, the behavioral components of anxiety can be measured in animal models with the powerful advantage of direct neurochemical correlation. Research into the biological substrates of anxiety has now gone beyond the traditional neurotransmitters to include neuropeptides within the central nervous system. More specifically, considerable evidence now exists for a role for

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corticotropin-releasing factor (CRF) in the neurobiology of anxiety (18,29,42). This 41-amino acid polypeptide is best known for its role in the peripheral response to stress through its actions on the hypothalamic–pituitary–adrenal axis (56). CRF and CRF receptors are also present in the amygdala and other extrahypothalamic brain areas, known to mediate the physiological, behavioral, and subjective experience of anxiety (16,17,47,50).

Various animal models of anxiety states such as the elevated plus-maze, acoustic startle, shock-induced freezing, and defensive withdrawal have been utilized to document the anxiogenic effects of CRF (18). It is doubtful that any single assay of animal behavior captures all of the components of the complex expression of anxiety. However, the defensive withdrawal test measures an animal's anxiety or fearfulness from an ethological perspective. The basis of this assay is the rat's natural tendency to explore its environment in the absence of perceived threat, and to retreat to an enclosed dark chamber when fearful (52).

The experiments presented here used a modified version of the defensive withdrawal test to examine several behavioral parameters. Initially the behavioral effects of direct injection of CRF into the lateral ventricle of naive rats were assessed. Subsequently we analyzed the ability of the monoamine oxidase inhibitor (MAO), phenelzine, which is used clinically to treat various anxiety disorders, to alter the behavioral effects of exogenous CRF. As phenelzine was chronically administered over 2–6 weeks, a saline control group was included in the study design to control for any potential anxiolytic effects that may have been detected in the assay as a result of habituation to the chronic stress of daily handling and subcutaneous (SC) injection.

In humans, panic attacks can be blocked and phobic avoidance reduced by the high-potency benzodiazepines such as alprazolam (4). These drugs reduce the concentration of CRF in the locus coeruleus, amygdala, and several cortical regions in the rat brain (39), and have an anxiolytic effect in a behavioral test sensitive to anxiolytic drugs, the elevated plus-maze (28). We hypothesized that other agents used clinically for panic and phobic anxiety, such as MAO inhibitor, phenelzine (32,54), might have an anxiolytic effect and attenuate the anxiogenic effects of exogenous CRF in the defensive withdrawal test. The effects of phenelzine on CRF content in the brain are not known. We further hypothesized that any changes in the behavioral response to exogenous CRF would be reflected in the binding characteristics of CRF in a major limbic forebrain structure, the amygdala.

The results suggest that chronic handling and saline injection caused a downregulation of CRF receptors in the amygdala, coincident with a decrease in defensive withdrawal behavior and responsiveness to CRF. However, in phenelzine-treated animals, behavioral habituation did not occur, and responsiveness to CRF was not reduced in spite of decreased CRF receptor binding in amygdala.

METHOD

Preparations of Animals

All procedures were approved by the West Virginia University Animal Care and Use Committee and conformed to the guidelines set forth by the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (Hilltop Labs, Scottsdale, PA), weighing 250–300 g, were anesthetized using pentobarbital (50 mg/kg, IP.). A 22-gauge stainless steel guide cannula/obturator assembly was stereotaxically implanted in the right lateral ventricle [−0.5 mm anteroposterior, +1.7 mm mediolateral from bregma, and 4.0 mm dorsoventral from the intraural line (41)]. The cannula was secured to the skull with 0-80 stainless steel screws and dental cement. Surgery was followed by at least a 7-day recovery period under single-housing conditions before behavioral testing. Animals were on a 12 L:12 D schedule (lights on at 0600 h) and were given food and water ad lib. Injections were made using a Hamilton syringe and polyethylene tubing through a 28-gauge cannula that extended 1.0 mm beyond the tip of the guide cannula. After behavioral testing, cannula placement in the lateral ventricle was visually confirmed by injection of toluidine blue immediately prior to sacrifice. Regional brain dissection was then performed and the tissues frozen in liquid nitrogen for the CRF receptor binding assay (see below).

Defensive Withdrawal Assay

The defensive withdrawal apparatus consisted of an open field that was 45 cm square and a defensive withdrawal chamber that was a 12 × 17 cm enclosed side compartment. Twenty-four hours prior to testing, animals were exposed to the lighted (100 lux) open field of the defensive withdrawal apparatus for 15 min without access to the withdrawal chamber itself. On the testing day, either 1.0 μg rat/human CRF (Bachem, Torrence, CA) or an equal volume (2 μl) of artificial cerebrospinal fluid (aCSF) was injected into the lateral ventricle. Animals were then returned to their home cages. After 25 min rats were placed in the withdrawal chamber with access to the field. Animals were videotaped using a Videomex-V (Columbus Instruments, Columbus, OH) activity counter for 15 min. Total time spent in the withdrawal chamber and total time in the lighted open field (all four paws out of the chamber) were measured. Latency (time from the start of the test to the first exit) was determined. Crossings (all four paws) from the defensive withdrawal chamber into the open field were recorded as exits. The number of rears (front paws raised) while in the open field was also recorded. All testing was conducted between 0800–1100 h.

Chronic Drug Treatment

After guide cannula placement (see above), two groups of animals received daily SC injections of 0.9% saline (0.1 ml) for 2–6 weeks. Two additional groups of rats received SC injections of phenelzine (sulfate salt, Sigma Chemical Co., St. Louis, MO; 3.0 mg/kg, 0.1 ml prepared in 0.9% saline) for 2–6 weeks. Daily injections were administered between 1400 and 1600 h. Twenty-four hours prior to behavioral testing, all animals underwent exposure to the open field of the defensive withdrawal apparatus. On the day of testing animals were injected ICV with either 1.0 μg CRF dissolved in 2 μl aCSF or 2 μl aCSF (vehicle control). Behavioral testing was conducted 25 min after CRF or aCSF administration, and 16–18 h after the last phenelzine or saline injection. Behavioral data were collected as described above.

^{125}I-Tyr^{0}-oCRF Binding

^{125}I-Tyr^{0}-oCRF (specific activity 2200 Ci/mmol) was purchased from Dupont–New England Nuclear (Boston, MA). Unlabeled rat/human CRF was purchased from Peninsula Laboratories (Belmont, CA). Standard reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Brains were obtained from treated male Sprague–Dawley rats by decapitation, followed immediately by dissection on ice. Amygdala was dissected as previously described (13), weighed, frozen in liquid nitrogen, and stored at −70°C until assayed.

Tissues were prepared for CRF receptor binding by the method described by Grigoriadis and De Souza (24), with significant modifications. Briefly, tissues (approximately 100–120 mg) were homogenized in 7 ml of buffer (Dulbecco's phosphate-buffered saline containing 10 mM $MgCl_2$, 2 mM EGTA, pH 7.0) with a Brinkmann Kinematica PT10-35 homogenizer. Membranes were sedimented by centrifugation at 30,000 × *g* for 14 min at 4°C. The pellet was rehomogenized in the same volume of the same buffer. After a second sedimentation of membranes at 30,000 × *g* for 14 min at 4°C, the pellet was resuspended to a concentration of 30 mg original wet weight of tissue per ml in assay buffer (Dulbecco's phosphate-buffered saline containing 10 mM $MgCl_2$, 2 mM EGTA, 0.15% bovine serum albumin, 0.15 mM bacitracin, 1.0 mM phenylmethylsulfonyl fluoride, pH 7.1) and kept on ice until use. ^{125}I-Tyr^0-oCRF binding was performed as previously described by De Souza (16) using 200 pM ^{125}I-Tyr^0-oCRF and 1 μM unlabeled rat/human CRF to define the nonspecific binding. Saturation curves were determined using concentrations of 50 to 800 pM ^{125}I-Tyr^0-oCRF. Protein determinations were performed according to the methods of Lowry et al. (33) using bovine serum albumin as the standard.

Data Analysis

Data were analyzed for statistically significant differences by two-factor ANOVA [chronic treatment (none, saline, or phenelzine), and anxiogenic challenge (2 μl aCSF or 1 μg CRF)]. When significant differences between groups were observed ($p < 0.05$), post hoc comparisons between relevant groups were done using Tukey's protected *t*-test. Values in figures and tables represent mean ± SEM.

RESULTS

The Acute Model: Defensive Withdrawal Following ICV CRF

To establish a baseline for the behavioral effects of ICV CRF, animals that had undergone no chronic treatment and no handling other than cannula implantation were challenged with 1.0 μg CRF or an equal volume of aCSF (2 μl) as an injection control. As has been reported by others (59), CRF produced an anxiogenic effect; animals receiving ICV CRF spent more time in the defensive withdrawal chamber, $F(1, 60) = 15.88$, $p = 0.0002$, compared to animals receiving an ICV injection of aCSF (Fig. 1). Latency (time to the first exit from the chamber), exits (total number of exits from the chamber), and rearing behavior (rears/min in the open field) were also measured (Table 1). In unhandled rats CRF caused a decrease in rears/min, indicative of an inhibition of exploratory behavior, and a decrease in exits, indicative of increased fear, but no effects on latency were detected.

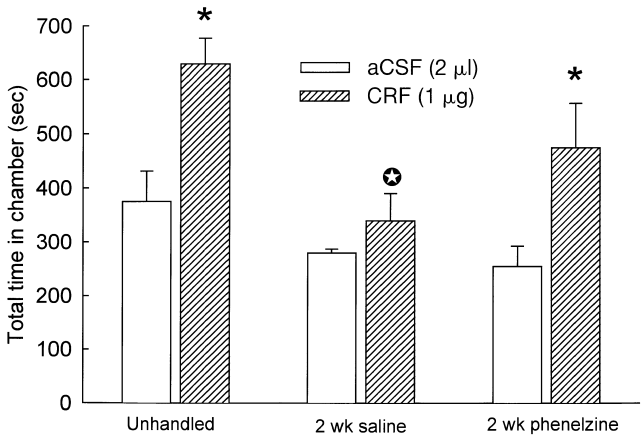

FIG. 1. Defensive withdrawal (total time in chamber) in response to 2 μl aCSF (ICV injection control) or 1 μg CRF (in 2 μl aCSF, ICV) in unhandled rats and following 2 weeks daily treatment with saline (0.9%, 0.1 ml, SC) or phenelzine (3 mg/kg, SC). Values are means ± SEM ($n = 9$–12). *Significant effect of CRF within treatment group, $p < 0.05$, Tukey's protected *t*-test; circled star: significant effect of handling and saline injection (vs. unhandled), $p < 0.05$, Tukey's protected *t*-test.

TABLE 1

ANXIOGENIC EFFECTS OF CRF IN UNHANDLED RATS AS MEASURED BY THE DEFENSIVE WITHDRAWAL TEST*

	Control (2 μl aCSF)	CRF (1 μg)
Latency (s)	9.3 ± 1.4†	9.5 ± 1.2
Total number of exits	16 ± 2	10 ± 2‡
Rears/min in field	5 ± 0.6	2 ± 0.5‡

*Animals were injected ICV with 2 μl aCSF (vehicle control) or 1 μg CRF 25 min prior to behavioral testing.

†Values are expressed as mean ± SEM ($n = 11$).

‡Significant effect of CRF (vs. aCSF), $p < 0.01$, Student's *t*-test.

Effects of Mild Chronic Stress

To control for adaptive changes that may have occurred within the CRF systems as a result of daily handling and SC injection, a group of rats were injected daily with 0.9% saline for 2–6 weeks. After 2 weeks of saline injection, the baseline defensive withdrawal in animals injected with aCSF (i.e., the ICV injection control) was unchanged compared to unhandled animals injected with aCSF, but the anxiogenic response to 1 μg CRF was abolished [Fig. 1; $F(1, 63) = 15.88$, $p = 0.0002$; $F(2, 63) = 6.65$, $p = 0.0025$]. Six weeks of daily handling and saline injection resulted in a significant reduction in baseline defensive withdrawal behavior (Fig. 2). Moreover, CRF (1.0 μg ICV) failed to elicit an anxiogenic response, i.e., no change in defensive withdrawal behavior, $F(1, 65) = 16.6$, $p = 0.0001$; $F(2, 65) = 25.18$, $p < 0.0001$. These results suggest that adaptation to a chronic mild stress decreased the responsiveness to CRF by 2 weeks that persisted through 6 weeks, and decreased the baseline withdrawal response at 6 weeks. Latency, exits, and rears after injection of aCSF or 1 μg CRF were not altered by chronic handling (data not shown).

Effects of Chronic Treatment With Phenelzine

Rats were treated once per day with 3.0 mg/kg (SC) phenelzine for 2 or 6 weeks. This dosing schedule has been shown to achieve >85% inhibition of both the A and B form of MAO (6). Evaluation of MAO activity in human subjects receiving therapeutic regimens of phenelzine suggests that favorable clinical responses are likely to occur when platelet MAO is inhibited by at least 85% (44). In the clinical setting, the antidepressant/anxiolytic effect of phenelzine is delayed for 2–3 weeks and may take up to 4–6 weeks (3). The 2- and 6-week treatment period before behavioral testing was based on this delayed onset of therapeutic effects seen in humans with depression and anxiety disorders.

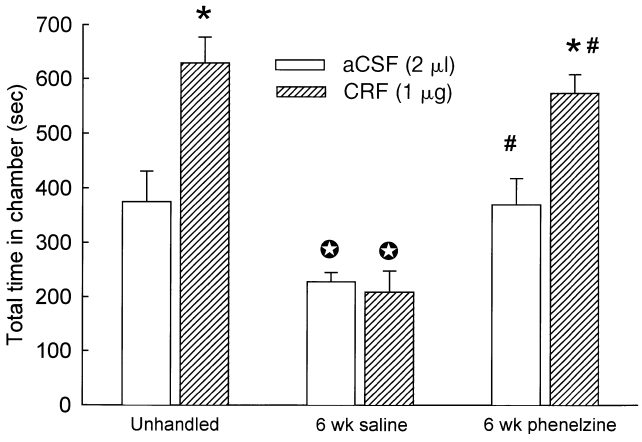

FIG. 2. Defensive withdrawal (total time in chamber) in response to 2 μl aCSF (ICV injection control) or 1 μg CRF (in 2 μl aCSF, ICV) in unhandled rats and following 6 weeks daily treatment with saline (0.9%, 0.1 ml, SC) or phenelzine (3 mg/kg, SC). Values are means ± SEM (n = 9–12). *Significant effect of CRF within treatment group, $p < 0.05$, Tukey's protected t-test; circled star: significant effect of handling and saline injection (vs. unhandled), $p < 0.05$, Tukey's protected t-test; #significant effect of phenelzine (vs. saline), $p < 0.05$, Tukey's protected t-test.

The baseline defensive withdrawal behavior of rats treated for 2 weeks with phenelzine did not differ significantly from rats treated for 2 weeks with saline. However, the response to CRF in phenelzine-treated rats was similar to the response in unhandled rats [Fig. 1; $F(1, 63) = 15.88$, $p = 0.0002$; $F(2, 63) = 6.65$, $p = 0.0025$], and thus significantly greater than the response to CRF in saline-treated rats.

Animals treated for 6 weeks with phenelzine exhibited more defensive withdrawal under baseline conditions (aCSF) and were more responsive to CRF compared to rats treated for 6 weeks with saline (Fig. 2). In fact, rats treated 6 weeks with phenelzine spent essentially the same amount of time in the defensive withdrawal chamber after receiving ICV aCSF as rats that had undergone no chronic treatment. Moreover, rats treated 6 weeks with phenelzine exhibited an anxiogenic response similar to unhandled animals when challenged with ICV CRF, $F(1, 65) = 16.6$, $p = 0.0001$; $F(2, 65) = 25.18$, $p < 0.0001$. Chronic phenelzine treatment did not affect latency, exits, or rears, or the CRF-induced changes in these behaviors (data not shown).

CRF Receptor Binding in the Amygdala

Saturation experiments using increasing concentrations of ^{125}I-Tyr^0-oCRF demonstrated kinetic parameters for receptor binding similar to those reported by De Souza for ^{125}I-Tyr^0-rat CRF (16). A representative saturation curve and Scatchard analysis is shown in Fig. 3. ^{125}I-CRF binding was saturable, reversible, and consisted of a single, high-affinity component with an apparent equilibrium dissociation constant (K_d) of 387 pM.

Amygdala from unhandled rats and rats chronically treated with saline or phenelzine was assayed for receptor binding using 200 pM ^{125}I-Tyr^0-CRF. This concentration of ligand provided high specific binding as a percent of total binding (> 50% specific binding). Animals that had undergone daily handling and saline or phenelzine injections for 2 or 6 weeks had more than a 50% decrease in CRF receptor

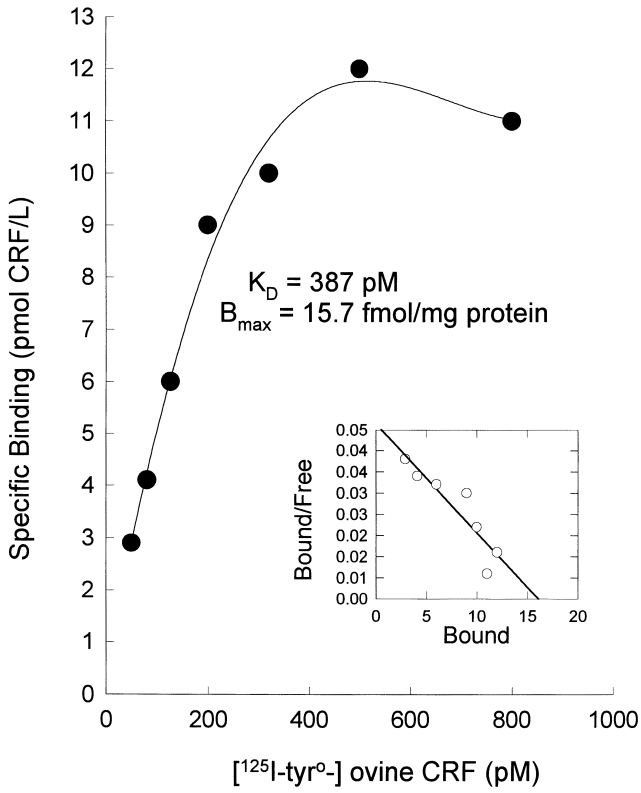

FIG. 3. Saturation kinetics of ^{125}I-Tyr^0-oCRF binding in rat brain. Binding assays were carried out as described in the Method section using concentrations of 50 to 800 pM ^{125}I-Tyr^0-oCRF. Assays were performed in triplicate in four separate experiments.

binding in the amygdala compared to unhandled controls, $F(4, 59) = 23.7$, $p < 0.0001$, Fig. 4). There were no significant differences in receptor binding between saline and phenelzine-treated rats.

DISCUSSION

We have observed that activation of CRF systems in the brain produces a reluctance to exit a defensive withdrawal chamber and explore an open field. These behavioral data are consistent with findings of other investigators who have used a similar defensive withdrawal test to measure anxiety in the rat (52). CRF neurotransmission within the hypothalamic–pituitary–adrenal axis is a dynamic and environmentally responsive system. In anticipation of similar plasticity within the extrahypothalamic CRF system, the present study controlled for adaptive changes that may have occurred in response to the mild stress of daily handling and SC injection that was required for the chronic administration of phenelzine. Defensive withdrawal following ICV injection of aCSF in rats that had undergone 6 weeks of handling was lower than that established in the unhandled controls. This finding is in agreement with many other studies that have demonstrated habituation to mild, predictable stressors in terms of the response of the hypothalamic–pituitary–adrenal axis and the sympathoadrenal response (20,36). Habituation to the mild chronic stress of handling and saline injection also fundamentally altered the anxiogenic effects of exogenous CRF. After 2 or 6 weeks of SC saline injection, the anxiogenic effect of CRF was abol-

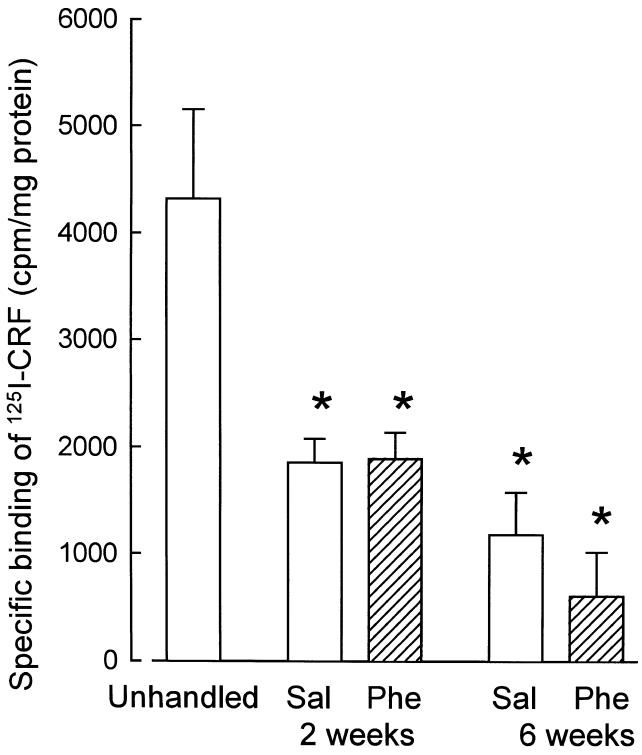

FIG. 4. Specific binding of ^{125}I-CRF in amygdala from control (unhandled, naive) rats ($n = 8$), and rats injected once daily with phenelzine (phe, 3 mg/kg, SC) or saline (sal, 0.9%, 0.1 ml, SC) for 2 or 6 weeks. Values are means ± SEM ($n = 12$). *$p < 0.05$ vs. unhandled control, Tukey's protected t-test. There were no significant differences between phenelzine and saline injection or between 2 and 6 weeks of treatment.

ished. This represents the first report that habituation to a chronic stress reduces the anxiogenic effect of CRF, as measured in the defensive withdrawal test. Others have shown that chronic stress reduces the anxiogenic efficacy of a benzodiazepine inverse agonist or an acute stressor as measured in the light:dark transition test (9). Moreover, habituation to handling also reduces the anxiolytic efficacy of benzodiazpines (7) and other anxiolytics (2) in the elevated plus-maze.

The neurochemical basis of habituation to mild stress has not been well defined. Loss of the anxiogenic effects of CRF in the defensive withdrawal paradigm could result from the downregulation of CRFergic synaptic transmission due to repetitive CRF release that would occur during the mild stress of daily handling and SC injection. Others have reported that chronic administration of CRF causes a desensitization to the behavioral effects of exogenous CRF (1), and downregulation of CRF receptors in the amygdala (25), and that chronic administration of cocaine, which mimics in many ways a chronic stress paradigm, also downregulates CRF receptors in both the amygdala and mesocortical brain regions (22). Although severe stressors, such as prolonged immobilization, appear to have no effect on CRF receptors in amygdala (26,27), we have found that the mild stress of daily saline injection causes a significant decrease in CRF binding in this brain region. The amygdala is a critical relay point for both behavioral and autonomic responses to stress (15). CRF injected into the amygdala has anxiogenic effects (19,31), and intra-amygdala injection of CRF antagonists blocks many behavioral responses to stress and fear-provoking stimuli (29,43,51). The marked reduction in CRF binding that we observed in this brain area after 2 or 6 weeks of handling could explain the attenuation of the fearful response to exogenous CRF.

The anxiogenic effects of CRF on latency, exiting, and rearing behavior were not significantly altered by chronic stress, indicating a differential effect on these three separate measures of anxiety. Differential effects on behavior in the defensive withdrawal test have been reported by others. For example, β-blockers, such as propranolol, reduce restraint stress-induced increases in the time in the chamber without altering increases in latency or rearing behavior (60). Although it is not possible to attribute specific brain function to individual components of the defensive withdrawal test, it is entirely consistent with the complex expression of anxiety that a differential response could emerge along the continuum of adaptation. Losing one of the three measures of anxiety in response to the anxiogenic challenge may represent a decreased sensitivity to CRF within a single brain area that mediates all of the behaviors in a graded manner. Another more likely possibility may be that these three behaviors are mediated by different brain areas, some of which undergo adaptation and others that do not.

A simple extrapolation of the clinical efficacy of phenelzine in social phobia and panic disorder led us to hypothesize that chronic phenelzine would decrease the anxiogenic effects of CRF. However, 2 or 6 weeks of phenelzine treatment failed to block the anxiogenic effects of CRF on defensive withdrawal compared to unhandled rats. In comparison to rats treated with saline for 2 or 6 weeks, where the anxiogenic effect of CRF was lost, phenelzine treatment actually enhanced, or maintained, the anxiogenic effect of CRF. Furthermore, comparison of the phenelzine-treated group to the saline-treated group revealed a failure to habituate to the chronic handling. In other words, baseline defensive withdrawal (after injection of aCSF) in the phenelzine-treated animals was not different from baseline withdrawal in unhandled rats.

Phenelzine's ability to prevent habituation to chronic handling and SC injections may be due to enhancement of monoaminergic neurotransmission. MAO is the major catabolic route for catecholamines and serotonin in neural tissues (10). Phenelzine irreversibly inactivates this enzyme. Following administration of MAO inhibitors brain monoamines, including norepinephrine, serotonin, and dopamine, become elevated (6). Over time, with continued phenelzine treatment, a downregulation of β-adrenoceptors is observed (38,40,49). In addition, chronic phenelzine enhances the responsiveness of the major noradrenergic cell group, the locus coeruleus (LC) by increasing LC discharge in response to phasic sensory stimuli and decreasing tonic discharge (14,57). This increase in the signal-to-noise ratio in the LC may be mediated by phenelzine-induced downregulation of inhibitory α_2-autoreceptors (30,35). These effects of phenelzine on the LC would tend to increase vigilance and heighten awareness of environmental stimuli (58), actions that could functionally antagonize habituation. It has been hypothesized that phenelzine's clinical efficacy is actually related to increased noradrenergic neurotransmission and not to receptor downregulation, because phenelzine is most efficacious in atypical depression, which may be a noradrenergic-deficient state (23). That phenelzine increases mRNA for tyrosine hydroxylase in the LC (8) would support a putative role in increasing noradrenergic function. Chronic phenelzine has been reported to increase aggression in neutral cage encounters (21), to have an anxiogenic effect in the social interaction test (28) and an anti-inactivity action in inescapable shock tests

(37). It lacks an anxiolytic effect in a typical anxiety measure like the elevated plus-maze (28) and the defensive withdrawal test (this report). Chronic phenelzine fails to produce effects similar to anxiolytics in measures of reticular-elicited or septal-driven hippocampal rhythmical slow activity, tests that are highly selective for anxiolytic drug action (61,62).

Taken together, this evidence suggest a possible explanation for the lack of anxiolytic effect of phenelzine on CRF-induced behaviors in the defensive withdrawal test. We have shown that the chronic stress of daily handling caused a downregulation of CRF receptors in the amygdala, which may be responsible for the loss of an anxiogenic effect of CRF, as well as the decrease in baseline defensive withdrawal. However, in the presence of phenelzine, we hypothesize an increase in noradrenergic activity, mediated through increased responsiveness of the LC, which could functionally antagonize the behavioral habituation to chronic stress and maintain baseline defensive withdrawal and the anxiogenic effect of CRF. The action of phenelzine to modify the effects of exogenous CRF is likely downstream of the action of chronic stress on CRF release in the amygdala, as chronic treatment with phenelzine did not prevent the downregulation of CRF binding in this brain region.

In summary, habituation to a mild chronic stress decreased baseline defensive withdrawal. Intraventricular administration of CRF produced an anxiogenic response as measured in the defensive withdrawal test, which was lost through exposure to mild chronic stress. Two or 6 weeks of daily handling and SC injection caused a downregulation of CRF receptors in the amygdala, which could account for the habituation and the loss of CRF-induced defensive withdrawal. Phenelzine treatment concurrent with mild chronic stress prevented habituation and maintained the anxiogenic effect of CRF in spite of the downregulation of CRF receptors in the amygdala. This effect of phenelzine, in light of its clinical efficacy to reduce panic and social phobia, invites further experimentation on the interactions of CRF and monoamines at the synaptic level.

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