Corticotropin Releasing Hormone Type 2 Receptors in the Dorsal Raphe Nucleus Mediate the Behavioral Consequences of Uncontrollable Stress

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Uncontrollable shock produces a constellation of behavioral changes that are not observed after equivalent escapable shock. These include interference with escape and potentiation of fear conditioning. The activation of corticotropin-releasing hormone (CRH) receptors within the caudal dorsal raphe nucleus (DRN) during inescapable tailshock (IS) has been shown to be critical for the development of these behavioral changes. CRH binds to two receptor subtypes, both of which are found in the DRN. The present set of studies examined which CRH receptor subtype mediates the effects of IS. Intra-DRN administration of the CRH_2 receptor antagonist anti-sauvagine-30 before IS dose-dependently blocked IS-induced behavioral changes; the CRH_1 receptor antagonist 2-methyl-4-(*N*-propyl-*N*-cycloproanemethylamino)-5-chloro-6-(2,4,6-trichloranilino)pyrimidine (NBI27914), administered in the same manner, did not. Moreover, the highly selective CRH_2 receptor agonist urocortin II (Ucn II) dose-dependently caused behavioral changes associated with IS in the absence of shock. Ucn II was effective at doses 100-fold lower than those previously required for CRH. The relationship between CRH_2 receptors and DRN 5-HT is discussed.

Key words: **corticotropin releasing hormone; corticotropin releasing hormone receptor; dorsal raphe nucleus; learned helplessness; serotonin; urocortin II**

Introduction

Situations in which organisms have no behavioral control over stressors produce a constellation of physiological and behavioral changes that do not occur if the stressor can be controlled, a phenomenon that has been called behavioral depression (Weiss et al., 1981) and learned helplessness (Maier and Seligman, 1976). When induced in animals, it includes many symptoms that overlap with depression and anxiety disorders (Porsolt et al., 1978; Sherman et al., 1979; Maier, 1984).

A variety of neural structures and transmitters are involved in the mediation of learned helplessness (Weiss et al., 1981; Maier et al., 1993). However, our laboratory has recently focused on changes in serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) as a key element. Uncontrollable stress [inescapable tailshock (IS)], relative to controllable stress (escapable tailshock), differentially activates 5-HT cells in the caudal DRN (Grahn et al., 1999b) and leads to large amounts of extracellular 5-HT within the DRN and its projection regions (Amat et al., 1998a,b; Maswood et al., 1998). These changes in DRN 5-HT have been shown to be critical to the production of learned helplessness (Maier et al., 1994, 1995a,b). The DRN is likely to receive a variety of inputs during uncontrollable stress, and a number have been delineated (Grahn et al., 1999a,c, 2000; Amat et al., 2001; Hammack et al., 2002). Corticotropin-releasing hormone (CRH) is particularly interesting in this regard.

CRH is a 41 residue polypeptide that plays a key role in integrating the endocrine (Vale et al., 1981), autonomic (Brown and Fisher, 1985), and behavioral (Koob et al., 1993) responses to stress. An involvement of CRH in the mediation of learned helplessness was first suggested by Ronan et al. (2000), who found that the intracerebroventricular administration of a large dose of CRH produced failure to learn to escape footshock 24 hr later, just as does IS. These findings were extended by Hammack et al. (2002), who found that DRN microinjection of a nonselective CRH antagonist before IS blocked the behavioral changes normally produced by IS, and that intra-DRN administration of CRH by itself produced IS-like behavioral changes 24 hr later. Furthermore, these effects were quite selective and were restricted to microinjection into the caudal DRN.

CRH and CRH-related peptides exert their biological activity by binding to two CRH receptor subtypes (DeSouza et al., 1985; Chalmers et al., 1995). The CRH_1 receptor is widely distributed throughout the CNS (Van Pett et al., 2000), whereas the CRH_2 receptor has a more limited distribution, primarily to subcortical regions (Chalmers et al., 1995). In addition, the two subtypes display quite different pharmacological profiles.

The study by Hammack et al. (2002) did not address the CRH receptor subtype within the DRN that mediates the effects of IS. The DRN contains an unusually high density of CRH_2 receptors, as well as CRH_1 receptors (Chalmers et al., 1995). The purpose of the reported experiments was to explore CRH_1 and CRH_2 recep-

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tor involvement in the DRN in the mediation of learned helplessness. The experiments determined whether (1) learned helplessness could be blocked by the intra-DRN microinjection of selective CRH_1 (NBI27914) and CRH_2 [anti-sauvagine-30 (ASV-30)] antagonists, and (2) whether learned helplessness could be induced by intra-DRN administration of the highly selective CRH_2 ligand urocortin II (Ucn II).

Materials and Methods

Animals

Male Sprague Dawley rats (Harlan Labs, Madison, WI) weighing 275–325 gm were used in all experiments. Rats were housed singly and maintained on a 12 hr light/dark cycle. Food and water were available *ad libitum*. Behavioral testing was performed between 8:00 A.M. and 12:00 P.M. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder.

Apparatus

Rats given IS were placed into 17.5 × 7.0 cm Plexiglas tubes. The rat's tail extended from the rear of the tube and was attached with tape to a Plexiglas rod. Electrodes were fixed to the tail, and computer-controlled 1.0 mA shocks were created by shock sources modeled after the Grason-Stadler Model 700 shock source (Grason-Stadler Inc., Madison, WI).

For behavioral testing, rats were placed into shuttleboxes measuring 46 × 20.7 × 20 cm. Scrambled 0.5 mA footshocks were delivered through stainless-steel grids on the floor of the apparatus. The shuttlebox was divided into halves by an aluminum wall containing an archway that allowed passage from one side to the other.

For locomotor activity, rats were placed in 30 × 30 × 30 cm Plexiglas boxes with walls that were striped with alternating 0.75 inch black and white electrical tape. The floor of each box was sanded Plexiglas covered with a wire mesh. A cable tie was loosely collared around the rat's neck, and a 2.0 × 2.5 cm piece of reflective tape was attached to the cable tie. This occurred the day before behavioral testing. The reflective tape was tracked by a CCD camera (Philips Inc., Lancaster, PA) mounted 1.5 m above the testing apparatus. The camera was connected to a computer, and data were collected via Chromotrack tracking software (Prototype Systems Ltd., Boulder, CO).

Surgery: cannulations

Rats were anesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) and implanted with guide cannulas into the region of the DRN or into the region of the central nucleus of the amygdala (CeA). Twenty-six gauge stainless-steel cannulas 13 mm long were implanted stereotaxically based on coordinates from the atlas of Paxinos and Watson (1986) and aimed 1.0 mm dorsal to the target region of the DRN or 3.0 mm dorsal to the area of the CeA to prevent damage to the areas. The bite bar was set at −3.5 mm, and the angle of approach was 0° (straight down). Coordinates 1.0 mm dorsal to the DRN were as follows: anteroposterior (AP), +1.0 mm; dorsoventral (DV), +4.3 mm; and mediolateral (ML), 0 mm, using interaural zero as a reference. Coordinates 3.0 mm dorsal to the CeA were as follows: AP, −2.5 mm; DV, −5.2 mm; and ML, ±4.2 mm, using the bregma as a reference.

Procedure

DRN NBI27914. One week after DRN cannulation, rats were randomly assigned to one of eight groups: IS plus 1.0 nmol of NBI27914, IS plus 0.5 nmol of NBI27914, IS plus 0.1 nmol of NBI27914, IS plus vehicle, home cage plus 1.0 of nmol NBI27914, home cage plus 0.5 nmol of NBI27914, home cage plus 0.1 nmol of NBI27914, or home cage plus vehicle. Thus, the design was a 2 (IS vs home-cage control) × 4 (drug dose) factorial. Each rat was handheld in a towel during the injection procedure. The stylet was removed, and rats were hand-injected through the guide cannula with the CRH_1 receptor antagonist NBI27914 (provided by Neurocrine Biosciences, San Diego, CA) or equivolume (0.5 μl) distilled water vehicle. The injector extended 1 mm below the end of the guide cannula into the DRN. Injectors were constructed of 33 gauge stainless-steel tubing (Small Parts Inc., Miami Lakes, FL) that was connected to a 50 μl Hamilton syringe with a length of polyethylene-20 tubing. The flow of drug was measured with a small air bubble created in the tubing. Injectors were left in place for 2 min to allow drug diffusion into brain tissue.

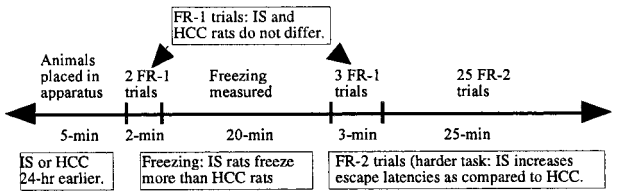

Figure 1. Procedure for measuring escape and fear behavior in the same subject.

Fifteen minutes after the injection, IS rats were given 100 5 sec tailshocks delivered on a 1 min variable-interval schedule. Home-cage rats were returned to their home cages after injection.

All subjects received behavioral testing 24 hr later. Both conditioned fear and shuttlebox escape learning were tested using a procedure described in detail previously (Maier et al., 1993). Freezing was measured for the first 5 min after placement in a shuttlebox. Each subject's behavior was scored every 8 sec as being either freezing or not freezing. Freezing was defined as the absence of all movement except that required for respiration. The observer was blind with regard to treatment condition, and inter-rater reliability has been calculated to be >0.92.

This observation period was followed by two footshocks, which could be terminated by crossing to the other side of the shuttlebox [fixed ratio-1 (FR-1) trials]. IS does not alter FR-1 shuttlebox escape latencies (Maier et al., 1993); therefore, IS and home-cage subjects are here exposed to shocks of equal duration. These two shocks were followed by a 20 min observation period in which freezing was scored. Previous work has indicated that this freezing is a measure of fear that has been conditioned to the contextual cues of the shuttlebox (Fanselow and Lester, 1988). This observation period was followed by three additional FR-1 escape trials and then 25 FR-2 escape trials. The subjects were required to cross to the other side and then back to terminate shock on the FR-2 trials, and it is here that IS-induced escape deficits are typically revealed. Each shock terminated after 30 sec if an escape response had not occurred. The testing procedure is schematized in Figure 1.

CeA NBI27914. To determine whether 1.0 nmol of NBI27914 was effective in our laboratory, 1.0 nmol of NBI27914 was injected into the CeA (0.5 nmol per side) immediately before fear conditioning in the shuttlebox as a positive control. One week after CeA cannulation, rats were injected with either NBI27914 or equivolume vehicle and placed in the shuttlebox. Rats subsequently received two FR-1 trials and were scored for freezing as described above.

DRN ASV-30. One week after DRN cannulation, rats were randomly assigned to one of eight groups: IS plus 1.0 nmol of ASV-30, IS plus 0.5 nmol of ASV-30, IS plus 0.1 nmol of ASV-30, IS plus saline vehicle, home cage plus 1.0 nmol of ASV-30, home cage plus 0.5 nmol of ASV-30, home cage plus 0.1 nmol of ASV-30, or home cage plus saline vehicle. Thus, the design here was also a 2 (IS vs home cage control) × 4 (drug dose) factorial. After injection, all procedures were identical to those described for the NBI27914 experiment.

DRN Ucn II. One week after DRN cannulation, rats were microinjected with 0.5 μl of either 0.021, 0.0021, or 0.00021 nmol (87, 8.7, or 0.87 ng, respectively) of Ucn II or saline vehicle. Immediately after injection, rats were placed into separate plastic bins in a different room from their home cages for 2 hr. Rats were then returned to their home cages. Twenty-four hours later, rats were behaviorally tested for conditioned fear and shuttlebox escape performance as described above.

DRN Ucn II and locomotor behavior. One week after DRN cannulation, rats were microinjected with 0.5 μl of either 0.021 nmol (87 ng) of Ucn II or saline vehicle. Immediately after injection, rats were placed into separate plastic bins in a different room from their home cages for 2 hr. Rats were then fitted with a homemade collar that contained a 2 × 2 cm surface made of reflective tape that was oriented dorsally. The collars were fitted securely but without causing obvious discomfort. Rats were then returned to their home cages. Twenty-four hours after injection, rats were placed in the testing boxes for 50 min to assess locomotor behavior, which was tracked by the computer software.

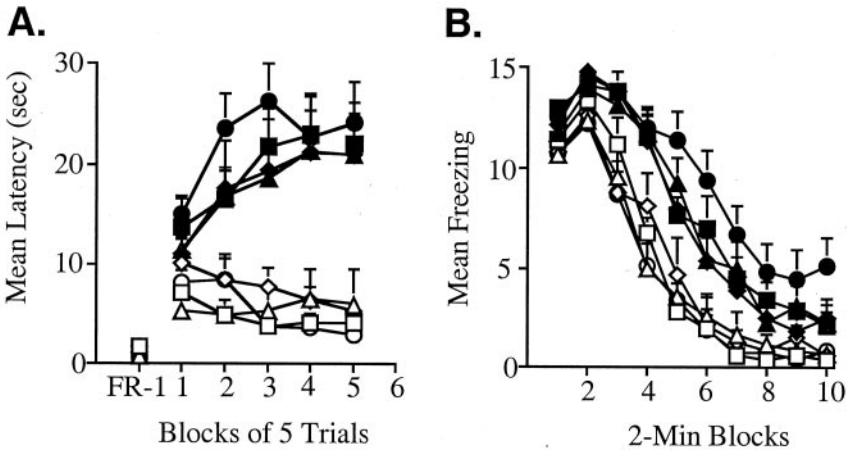

Figure 2. *A*, Mean shuttlebox escape latencies for FR-1 trials and five blocks of FR-2 trials. Rats either received IS or were left in their home cages 24 hr previously. Rats also received an intra-DRN injection of 0.1, 0.5, or 1.0 nmol of the CRH_1 receptor antagonist NBI27914 or vehicle 15 min before IS treatment. *B*, Mean number of 8 sec periods in which freezing occurred across 2 min blocks, after two shocks in a shuttlebox. Rats either received IS or were left in their home cages 24 hr previously. Rats also received an intra-DRN injection of 0.1, 0.5, or 1.0 nmol of the CRH_1 receptor antagonist NBI27914 or vehicle 15 min before IS treatment. *Open squares*, Home cage plus vehicle; *open triangles*, home cage plus 0.1 nmol of NBI27914; *open diamonds*, home cage plus 0.5 nmol of NBI27914; *open circles*, home cage plus 1 nmol of NBI27914; *filled squares*, IS plus vehicle; *filled triangles*, IS plus 0.1 nmol of NBI27914; *filled diamonds*, IS plus 0.5 nmol of NBI27914; *filled circles*, IS plus 1 nmol of NBI27914.

Histology

To verify cannula placements, cannulated rats were anesthetized and injected through the guide cannula with Evans Blue dye (1 μl). Rats were perfused after 15 min of dye diffusion, and their brains were removed and fixed in a 10% formalin and 30% sucrose solution. Brains were then sectioned on a cryostat and stained with cresyl violet. Cannula verifications of the sections were conducted under a light microscope.

Statistical analysis

Data were analyzed with repeated-measures ANOVA and followed with a Newman–Keuls analysis (α set at 0.05), which made all possible pairwise comparisons.

Results

DRN NBI27914

Injecting the CRH_1 receptor antagonist NBI27914 into the DRN before IS did not alter the interference with FR-2 escape responding (Fig. 2*A*) or potentiated fear conditioning (Fig. 2*B*) normally observed 24 hr later at any dose tested. For FR-2 escape latencies, there was an effect of group ($F_{(7,47)} = 8.104$; $p < 0.05$), an effect of trials ($F_{(4,188)} = 6.157$; $p < 0.05$), and a significant interaction between group and FR-2 trials ($F_{(28,188)} = 3.782$; $p < 0.05$). These significant effects indicated that IS increased escape latencies above home-cage treatment. Newman–Keuls analysis revealed that there was no effect of any dose of NBI27914. All IS groups differed from all home-cage groups, but IS groups administered each dose of NBI27914 did not differ from IS groups administered vehicle, nor did they differ from each other. Moreover, there was no effect of NBI27914 in home-cage rats, because there were no significant differences between home-cage rats given any dose of NBI27914 or vehicle. For freezing behavior, there was a significant effect of group ($F_{(7,45)} = 7.042$; $p < 0.05$), a significant effect of 2 min blocks ($F_{(9,405)} = 305.089$; $p < 0.05$), and a significant interaction between group and 2 min blocks ($F_{(63,405)} = 7.644$; $p < 0.05$). Again, these significant effects indicated that IS potentiated freezing above home-cage treatment; however, Newman–Keuls analyses revealed that there was no effect of any dose of NBI27914. All IS groups differed from all home-cage groups, but IS groups administered each dose of NBI27914 did not differ from IS groups administered vehicle, nor did they differ from each other. Moreover, there was no effect of NBI27914 in home-cage rats, because there were no significant differences between home-cage rats given any dose of NBI27914 or vehicle.

CeA NBI27914

Cannula placements for rats injected with NBI27914 into the CeA are shown in Figure 3. Injecting the CRH_1 receptor antagonist NBI27914 into the CeA before fear conditioning suppressed freezing behavior measured after two shocks in a shuttlebox (Fig. 4). There was an effect of drug treatment ($F_{(1,10)} = 18.823$; $p < 0.05$), an effect across the 10 2 min blocks of freezing ($F_{(9,90)} = 18.851$; $p < 0.05$), and an interaction between drug treatment and 2 min blocks of freezing ($F_{(9,90)} = 2.282$; $p < 0.05$).

DRN ASV-30

Cannula placements for rats injected with 1 nmol of ASV-30 into the DRN are shown in Figure 5. Injecting the CRH_2 receptor antagonist ASV-30 into the DRN before IS dose-dependently blocked the interference with FR-2 escape responding (Fig. 6*A*) and potentiated fear conditioning (Fig. 6*B*) normally observed 24 hr later. When ASV-30 was administered into the DRN before IS, the 0.5 nmol dose suppressed, and the 1.0 nmol dose blocked, the interference with escape responding normally observed 24 hr later, and the same pattern was exhibited by freezing behavior. For FR-2 escape latencies, there was an effect of group ($F_{(7,50)} = 10.910$; $p < 0.05$), an effect of trials ($F_{(4,200)} = 2.387$; $p < 0.05$), and a significant interaction between group and FR-2 trials ($F_{(28,200)} = 3.624$; $p < 0.05$). Newman–Keuls analyses revealed that there was a significant difference between the IS plus vehicle group and all IS groups receiving a dose of ASV-30. In addition,

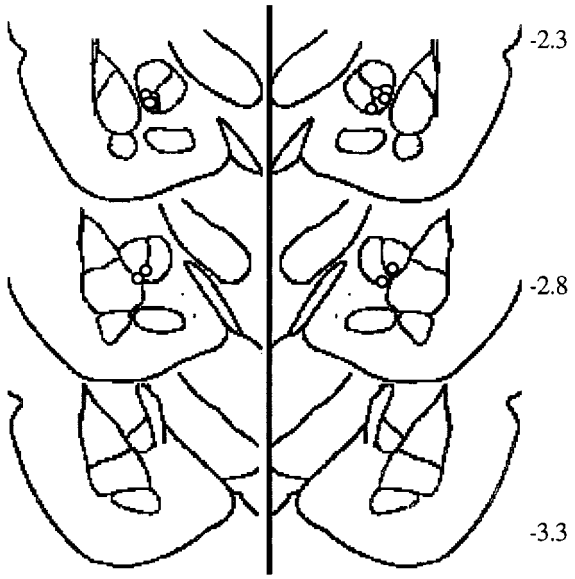

Figure 3. CeA injection placements for rats injected with 0.5 nmol of NBI27914 per side. Each *circle* represents the center of one dye injection.

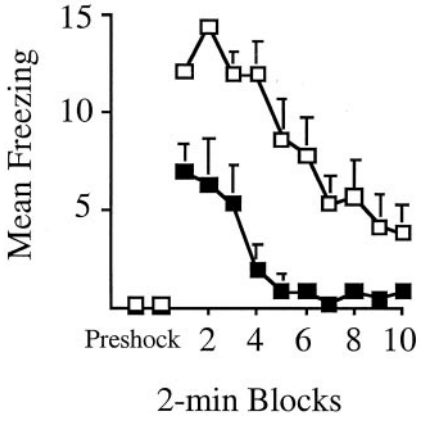

Figure 4. Mean number of 8 sec periods in which freezing occurred across 2 min blocks, after two shocks in a shuttlebox. Rats received 0.5 nmol of NBI27914 per side or equivolume vehicle into the CeA 15 min before the two shocks. Although NBI27914 did not affect IS-induced behavioral changes when injected into the DRN, it suppressed fear conditioning when injected into the amygdala. *Filled squares*, NBI27914; *open squares*, vehicle.

the IS plus vehicle group differed significantly from all home-cage groups. The IS plus 0.1 nmol of ASV group differed significantly from the IS plus 0.5 nmol of ASV and IS plus 1.0 nmol of ASV groups, and the latter two groups did not differ from each other or from any home-cage group. For freezing behavior, there was a significant effect of group ($F_{(7,50)} = 11.683$; $p < 0.05$), a significant effect of 2 min blocks ($F_{(9,450)} = 475.264$; $p < 0.05$), and a significant interaction between group and 2 min blocks ($F_{(63,450)} = 6.836$; $p < 0.05$). Similarly, Newman–Keuls analysis revealed that there was a significant difference between the IS plus vehicle group and all IS groups receiving a dose of ASV-30 except for the 0.1 nmol dose. In addition, the IS plus vehicle group significantly differed from all home-cage groups. The IS plus 0.1 nmol of ASV group significantly differed from the IS plus 0.5 nmol of ASV and IS plus 1.0 nmol of ASV groups, and the latter two groups did not differ from each other or from any home-cage group.

DRN Ucn II

Ucn II injected into the caudal DRN dose-dependently increased FR-2 escape latencies (Fig. 7*A*) and conditioned fear (Fig. 7*B*) 24 hr later. For FR-2 escape latencies, there was an effect of drug ($F_{(3,27)} = 3.395$; $p < 0.05$) and an interaction between drug and trials ($F_{(12,\ 108)} = 3.310$; $p < 0.05$). For freezing behavior, there was a reliable effect of drug ($F_{(3,26)} = 13.771$; $p < 0.05$) and an interaction between drug and time ($F_{(27,234)} = 2.158$; $p < 0.05$). The 0.021 nmol group was significantly different from all other groups.

DRN Ucn II and locomotor behavior

Ucn II injected into the caudal DRN did not affect locomotor behavior tested 24 hr later (Fig. 8). There was not even a marginally significant effect of drug ($F_{(1,14)} = 0.967$; $p > 0.05$). Although there was an effect of trials ($F_{(9,126)} = 3.176$; $p < 0.05$), there was no interaction between drug and trials ($F_{(9,126)} = 0.473$; $p > 0.05$).

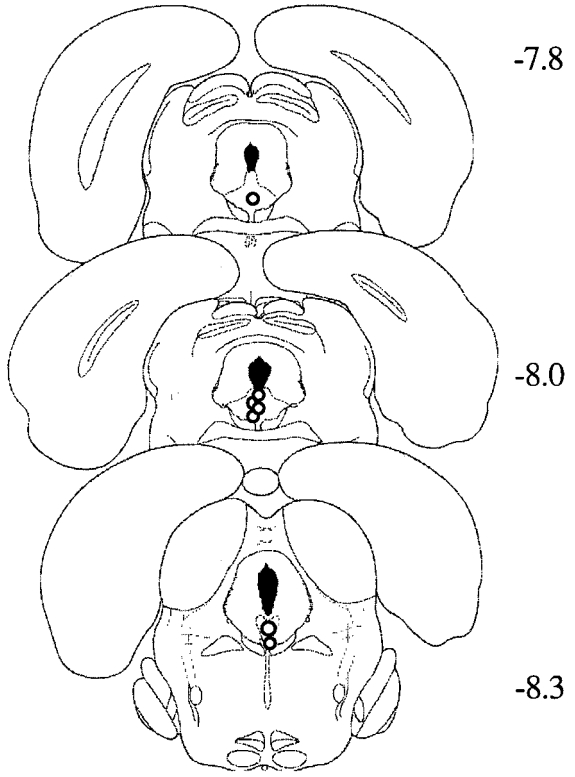

Figure 5. DRN injection placements for rats injected with 1 nmol of anti-sauvagine-30. Each *circle* represents the center of one dye injection.

Discussion

The present results further support the notion that CRH (Ronan et al., 2000), and CRH within the DRN (Hammack et al., 2002), play a key role in mediating the behavioral consequences of uncontrollable stressors. It is unknown whether the CRH or another CRH-related peptide that is involved is intrinsic to the DRN or derives from projections to the DRN, but it can be noted that the

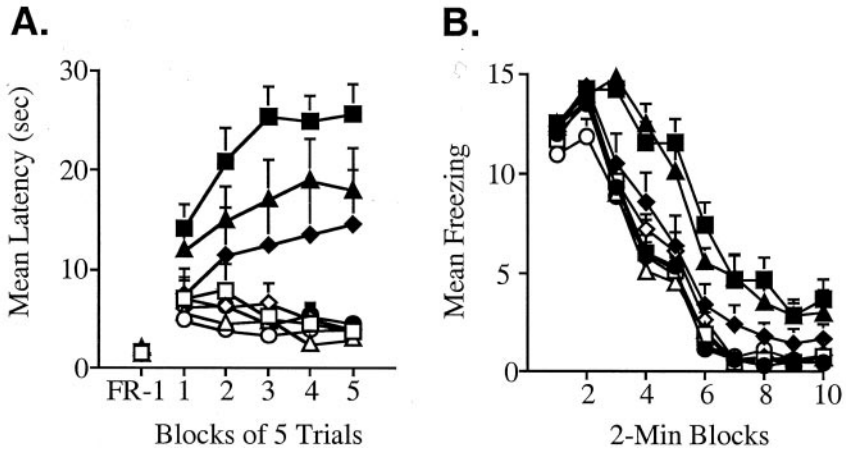

Figure 6. *A*, Mean shuttlebox escape latencies for FR-1 trials and five blocks of FR-2 trials. Rats received either inescapable shock or were left in their home cages 24 hr previously. Rats also received an intra-DRN injection of 0.1, 0.5, or 1.0 nmol of the CRH_2 receptor antagonist anti-sauvagine-30 or vehicle 15 min before IS treatment. *B*, Mean number of 8 sec periods in which freezing occurred across 2 min blocks, after two shocks in a shuttlebox. Rats received either inescapable shock or were left in their home cages 24 hr previously. Rats also received an intra-DRN injection of 0.1, 0.5, or 1.0 nmol of the CRH_2 receptor antagonist anti-sauvagine-30 or vehicle 15 min before IS treatment. *Open squares*, Home cage plus vehicle; *open triangles*, home cage plus 0.1 nmol of ASV-30; *open triangles*, home cage plus 0.5 nmol of ASV-30; *open circles*, home cage plus 1 nmol of ASV-30; *filled squares*, IS plus vehicle; *filled triangles*, IS plus 0.1 nmol of ASV-30; *filled diamonds*, IS plus 0.5 nmol of ASV-30; *filled circles*, IS plus 1 nmol of ASV-30.

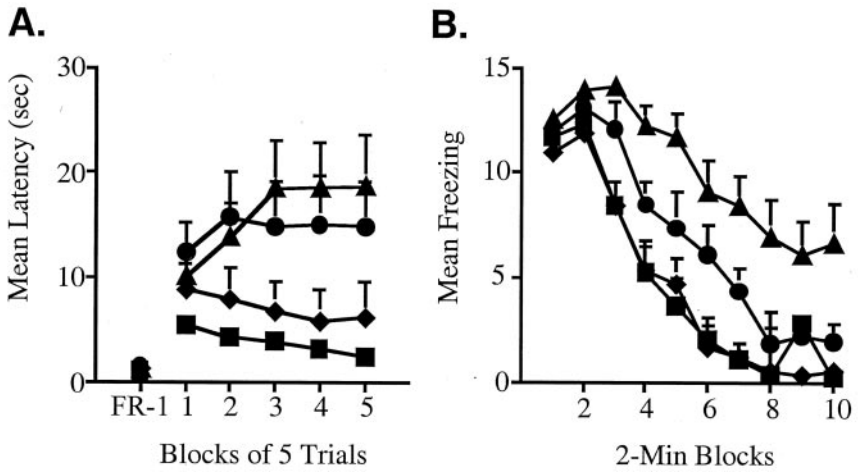

Figure 7. *A*, Mean shuttlebox escape latencies for FR-1 trials and five blocks of FR-2 trials. Rats either received 0.021 nmol (87 ng), 0.0021 nmol (8.7 ng), or 0.00021 nmol (0.87 ng) of the CRH_2 receptor agonist Ucn II or saline vehicle into the DRN 24 hr previously. *B*, Mean number of 8 sec periods in which freezing occurred across 2 min blocks, after two shocks in the shuttlebox. *Filled squares*, Vehicle; *filled diamonds*, 0.00021 nmol of Ucn II; *filled circles*, 0.0021 nmol of Ucn II; *filled triangles*, 0.021 nmol of Ucn II.

DRN receives CRH projections from a variety of forebrain and limbic structures, including the amygdala and bed nucleus of the stria terminalis (Swanson et al., 1983; Sakanaka et al., 1986). In addition, the CRH-related peptides Ucn I (Bittencourt et al., 1999), Ucn II (Reyes et al., 2001), and Ucn III (Lewis et al., 2001) mRNAs are localized in regions that are known to project to the DRN. However, whether the projections from these regions to the DRN contain Ucn II or Ucn III is unknown.

Although the present data do not indicate the source of CRH or other CRH-related peptide involvement, they do suggest that DRN CRH_2 receptors within the DRN mediate learned helplessness. ASV-30, which is 100- to 1000-fold selective for CRH_2 (Higelin et al., 2001), dose-dependently blocked both the interference with escape learning and potentiation of fear conditioning produced by IS. The highly selective CRH_1 antagonist NBI27914 (Chen et al., 1996) had no effect whatsoever at the same molar doses. Moreover, the NBI27914 doses were well within the effective range, given the intracerebroventricular doses that have been used (Baram et al., 1997; Martinez et al., 1998; Pelleymounter et al., 2000), and were effective in suppressing fear conditioning when injected into the CeA. Furthermore, the intra-DRN administration of Ucn II induced both poor escape learning and potentiation of fear conditioning 24 hr later, just as does IS. Ucn II was effective at 100–1000 lower molar concentrations than required for CRH to produce these effects (Hammack et al., 2002), and Ucn II has been estimated to be 100-fold more active at the CRH_2 receptor than is CRH (Reyes et al., 2001). Importantly, Ucn II has little if any activity at the CRH_1 receptor in either binding (Reyes et al., 2001) or cAMP production (Lewis et al., 2001) assays.

Although, Ucn II has been shown to suppress locomotor activity mildly (Valdez et al., 2002), there has been no indication that it would suppress activity 24 hr later. Nevertheless, the present experiments assessed whether the dose of Ucn II used here would suppress motor activity 24 hr later, and it did not. Therefore, the effects of Ucn II on shuttle escape 24 hr later could not be attributed to a general decrease in locomotor activity.

The presence of high densities of CRH_2 receptors within the DRN (Chalmers et al., 1995) is consistent with the possibility that CRH_2 rather than CRH_1 receptors within the DRN are critical in the production of learned helplessness. However, this conclusion might seem to be at odds with Mansbach et al. (1997) and Takamori et al. (2001), who reported that peripheral administration of CRH_1-selective nonpeptide antagonists before IS reduced IS-induced escape failure. There are at least two possibilities. First, CRH_1 receptors outside the DRN could also be involved in the mediation of the behavioral consequences of IS. Learned helplessness is mediated by a neural circuit that involves numerous structures and transmitters, with the DRN being only one, but perhaps a key, nodal point. Because the CRH_1 receptor has a wide distribution, this receptor could be involved at other points in the circuit and the Mansbach et al. (1997) and Takamori et al. (2001) studies used systemic drug administration. Second, the behavioral procedures used by Mansbach et al. (1997) and Takamori et al. (2001) were qualitatively different from those used here. Indeed, Deak et al. (1999) showed that the CRH_1-selective antagonist antalarmin did not block IS-induced escape failure using procedures identical to those presented here.

A predominant role within the DRN for the CRH_2 receptor in the mediation of learned helplessness aids in the explanation of some aspects of the data concerning the effects of CRH within this paradigm. Both Ronan et al. (2000) and Hammack et al. (2002) found that very large doses of CRH were needed to mimic the usual effects of IS. Ronan et al. (2000) found that 10.0 μg of intracerebroventricular CRH was required to produce escape failure 24 hr later, whereas Hammack et al. (2001) reported that two doses of 10.0 μg of intracerebroventricular rat/human (r/h) CRH were required. In contrast, behavioral effects presumably mediated by CRH_1 receptors have been produced using $\leq$1.0 μg intracerebroventricularly (Lee and Davis, 1997). Consistent with the requirement of these large intracerebroventricular doses, Hammack et al. (2001) reported that the intra-DRN microinjec-

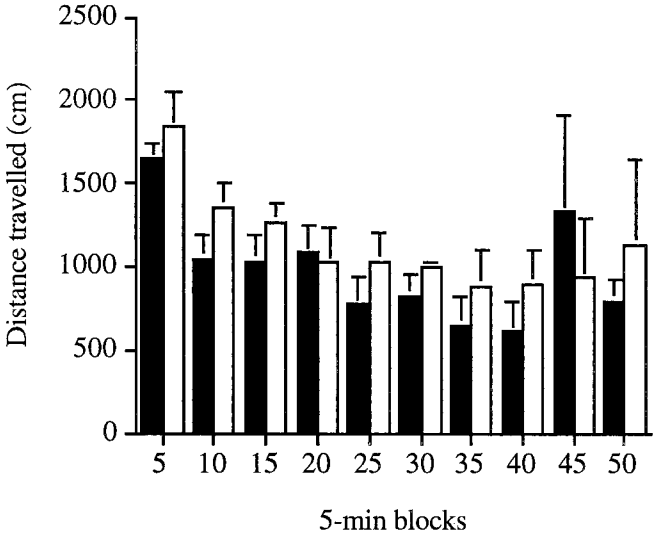

Figure 8. At 24 hr after 0.021 nmol (87 ng) of Ucn II or equivolume vehicle injection into the DRN, locomotor activity was scored in 5 min blocks for 50 min. Ucn II injection did not reliably alter locomotor activity tested 24 hr after injection. *White columns*, Ucn II; *black columns*, vehicle.

tion of between 0.5 and 1.0 μg of r/h CRH was needed to produce escape failures and potentiated fear conditioning 24 hr later. As a comparison, intra-DRN doses of CRH in the 1.0–10.0 ng range inhibit DRN 5-HT electrical activity, a phenomenon likely mediated by CRH_1 receptors in that it is blocked by antalarmin (Kirby et al., 2000). CRH binds preferentially to the CRH_1 receptor (Lovenberg et al., 1995), and so CRH_2 receptor mediation of learned helplessness would account for the large doses of CRH required to induce it. As noted above, much lower doses of Ucn II, which preferentially binds CRH_2 receptors, were required. These data suggest that the endogenous ligand responsible for DRN CRH_2 receptor activation during uncontrollable stress might not be CRH, but rather a peptide more selective to the CRH_2 receptor such as Ucn II or Ucn III.

The present data are also of potential relevance to an issue concerning whether CRH within the DRN excites or inhibits 5-HT neurons. Clearly, the implication here is that intra-DRN CRH mimics the behavioral effects of IS because it activates 5-HT neurons. This is because (1) IS does activate DRN 5-HT neurons (Maswood et al., 1998; Grahn et al., 1999b), (2) the behavioral effects of IS are mimicked by intra-DRN administration of other agents that do activate 5-HT neurons (Maier et al., 1995b; Grahn et al., 1999a), and (3) the intra-DRN administration of agents that inhibit 5-HT activity blocks the behavioral effects of IS (Maier et al., 1995a). However, low doses of intra-DRN and intracerebroventricular CRH have been reported to inhibit DRN 5-HT electrical activity (Price et al., 1998; Kirby et al., 2000) and 5-HT efflux in DRN projection regions (Price and Lucki, 2001). Interestingly, as the CRH dose was increased in these studies, the inhibitory effects decreased; with additional increases in dose, the effects of CRH tended to become excitatory. This pattern is consistent with the idea proposed by Kirby et al. (2000) that CRH_1 receptors might mediate predominantly inhibitory effects on 5-HT neurons within the DRN, whereas CRH_2 receptor activation leads to excitatory effects. Indeed, Price and Lucki (2001) reported that r/h CRH lacked inhibitory effects at the same dosages at which ovine CRH (oCRH) exerted strong inhibition. This is noteworthy because oCRH has a higher selectivity for the CRH_1 receptor than does r/h CRH (Lovenberg et al., 1995).

The present data are consistent with other recent reports implicating the CRH_2 receptor in the mediation of anxiety (Ho et al., 2001; Takahashi et al., 2001; Bakshi et al., 2002), although they are at odds with other reports suggesting an anxiolytic role for the CRH_2 receptor (Bale et al., 2000, 2002; Kishimoto et al., 2000). These latter studies have shown that CRH_2 receptor knock-out mice show increased levels of anxiety on several tests, suggesting an anxiolytic role for CRH_2 receptors. However, the interpretation of such data are difficult because of the possibility that compensatory mechanisms may change the behavioral phenotype of the mature mice. Furthermore, in studies in which CRH_2 receptors were inactivated by antagonists (Takahashi et al., 2001; Bakshi et al., 2002) or antisense oligonucleotides (Ho et al., 2001), anxiety-like behavior was generally suppressed, suggesting an anxiogenic role for the receptor. In any case, the present experiments address only the role of the CRH_2 receptor in the DRN. This is a very different issue than the net effect of CRH_2 receptor activation across the brain. The role of the CRH_2 receptor in anxiety is likely complicated, and the site of action may be critical. For example, antagonism of CRH_2 receptors in the lateral septum suppresses shock-induced freezing (Bakshi et al., 2002).

Exposure to uncontrollable, relative to controllable, stressors induces behavioral changes characteristic of anxiety (Short and Maier, 1993). The DRN and 5-HT projections from the DRN have been argued to be important in the production of anxiety (Graeff et al., 1996), and CRH_2 receptors in this region may play an important role in the activation of these neurons during anxiety-arousing circumstances such as exposure to uncontrollable stressors.

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