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Corticotropin-releasing factor potentiates acoustic startle in rats: Blockade by chlordiazepoxide

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Abstract. A series of experiments was performed to investigate the effects of corticotropin-releasing factor (CRF) on the amplitude of the acoustic startle response (ASR) in rats. Intracerebroventricular (ICV) administration of 1 μg rat CRF significantly potentiated acoustic startle amplitude; these effects were reversed in a dose-dependent manner by pretreatment with the benzodiazepine chlordiazepoxide (CDP). Doses of CDP that anatgonized CRF-potentiated ASR did not lower startle baseline or antagonize amphetamine- or strychnine-potentiated ASR. These results suggest that CRF has "anxiogenic" properties and may serve as a neuroendocrine modulator of stress-enhanced behaviors.

Key words: Corticotropin-releasing factor – Startle – Chlordiazepoxide – Anxiety – Strychnine – Amphetamine

CRF is a 41 amino-acid polypeptide released within the median eminence from cells in the paraventricular nucleus of the hypothalamus, which stimulates the release of ACTH and β-endorphin from the anterior pituitary (Rivier et al. 1981; Vale et al. 1981). In addition to its endocrine-activating properties, there is much evidence that CRF may have direct neurotropic actions. CRF-immunoreactive cells and fibers have been found in a wide distribution throughout the rat CNS (Swanson et al. 1983), and CRF has been shown to increase firing frequencies of cells within the locus coeruleus in vivo (Valentino et al. 1983), and to activate cells in hippocampal slice preparations (Aldenhoff et al. 1983). CRF administered intracerebroventricularly (ICV) produces a dose-dependent activation of EEG, including interictal spikes in the amygdala and hippocampus (Ehlers et al. 1983), and high doses produce kindling-like seizures in the amygdala that cross-sensitize with electrically-induced seizure activity (Weiss et al. 1984).

In addition to its endocrine and neurotropic properties, CRF has been shown to stimulate behavioral changes in the rat which mimic behaviors normally exhibited during conditions of high stress. When administered ICV in rats placed in a novel open field environment, CRF potentiates the effects of "novelty": treated rats exhibit movement restricted to the outer walls of the environment, together with decreased exploration and increased grooming (Britton et al. 1982). In a familiar environment, CRF-treated rats show evidence of intense activation: locomotion, rearing and sniffing are increased greatly for several hours (Sutton et al. 1982). These behaviors probably result from the effects of CRF within the CNS, since they are not blocked by hypophysectomy (Eaves et al. 1985), and are not produced by peripheral injections of CRF in doses greater by orders of magnitude (Sutton et al. 1982). In "conflict" paradigms where a food-deprived rat is trained to press a lever to receive both food and shock, CRF decreases responding, an effect indicative of "anxiogenic" properties which is reversed by benzodiazepines (Britton et al. 1985). Thus, CRF is found throughout the brain, and is believed to produce neuroendocrine, electrophysiological and behavioral changes in the rat consistent with an exaggeration of the "stressfulness" of the environment.

The acoustic startle reflex (ASR) is an easily quantified contraction of the skeletal musculature in response to an intense acoustic stimulus. In rats, the ASR has been shown to be sensitive to states of stress or fear, since ASR amplitude is enhanced by drugs (such as piperoxane and yohimbine) that have anxiogenic properties in humans (Davis and Astrachan 1981) and when the acoustic stimulus is presented during another stimulus (light, for example) that has previously been paired with shock (Brown et al. 1951). This "potentiated startle" is enhanced by piperoxane and yohimbine (Davis et al. 1979), and is attenuated by drugs (such as diazepam, flurazepam, morphine, alcohol and sodium amytal) that have anxiolytic properties in humans (Chi 1965; Miller and Barry 1960; Davis 1979a, b). The ASR and "potentiated startle" paradigm have thus been used as model systems to study how drugs alter stress and fear.

As reviewed by Davis (1980), the acoustic startle paradigms offer several distinct interpretative advantages not found in other models of anxiety. In contrast to open field measurements, startle is automated and not subject to observational variability or bias. The startle reflex is elicited under tight stimulus control, and is suitable for parametric analysis using different stimulus intensities, interstimulus intervals, pre-pulse conditions and background white noise levels to generate desired response characteristics. Unlike responses measured in the operant conflict paradigm,

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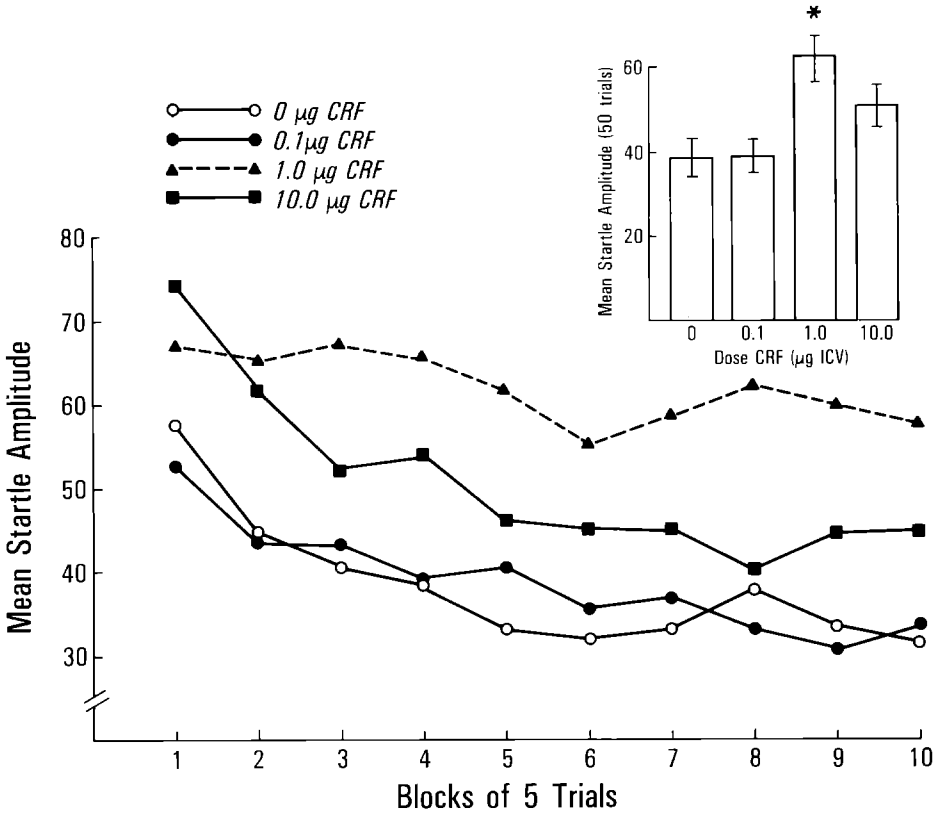

Fig. 1. Acoustic startle amplitude in rats following ICV infusion of CRF (0, 0.1, 1.0 or 10.0 μg). *Insert histogram* indicates mean startle amplitude over 50 trials. $*P<0.001$, Newman-Keuls individual means comparison

acoustic startle is controlled by a well-characterized neural circuit (Davis et al. 1982), and thus permits an analysis of the neural substrates of anxiety (Mondlock and Davis 1985). Since the startle reflex involves no operant response, training is not required and interpretative problems of state dependency are avoided. Finally, the ASR can be quantified in numerous species, including humans, and thus predictions generated from studies of acoustic startle in rats can be directly tested in a clinical setting.

The purpose of the present study was to examine the behavioral effects of CRF using a reflex response that is known to be sensitive to states of stress or fear. The approach was first to evaluate the effects of CRF on baseline startle amplitude, and then to determine whether CRF-induced changes in ASR amplitude could be attributed to "anxiogenic" or fear-enhancing properties of CRF by attempting to antagonize these effects with the anxiolytic drug chlordiazepoxide.

Experiment I

If the behavioral changes produced by CRF in the rat reflect a state of elevated stress or fear, then CRF should potentiate the amplitude of the ASR. In the first experiment, rats were tested for their ASR following ICV administration of CRF in a dose range (0, 0.1, 1.0 and 10.0 μg) known to produce behavioral activation and evidence of "fear" in other behavioral measurements (Sutton et al. 1982).

Material and methods

Animals. Ninety-two male Wistar rats (200–220 g, Charles River Laboratories) were housed in groups of three, exposed to a normal 12-h light-dark cycle, with free access to food and water. Each animal was handled for 5 min within 3 days of shipment arrival, before any habituation, surgical or testing procedures were undertaken.

Surgery. For CRF experiments, rats were implanted with a stainless steel cannula aimed at the lateral ventricle. Rats were anesthetized with pentobarbital (50 mg/kg), secured in a Kopf stereotaxic instrument, and a 7-mm stainless steel guide tube (23 ga) was aimed 1 mm above the lateral ventricle and secured to the skull with two stainless steel screws and Silux dental cement. Coordinates were (toothbar+5 mm): AP −0.6 (Bregma), L 2.0, DV −3.2 (skull). A 7 mm wire stylet filled the cannula.

Apparatus. The apparatus used was the SRLAB from SPSG, La Jolla, CA. A single stabilimeter cage consisting of a cylindrical Plexiglas tube held within a rigid frame by rubber stoppers was housed in a sound attenuation chamber. Reflex amplitude and peak latency were measured during the 200-ms interval following presentation of the noise burst (118 dB, A scale). Background white noise was 59 dB.

Procedure. One week after surgery and 4 days before testing, each animal ($n=32$) was placed in the stabilimeter cage for 5 min, and then presented with five tones at a 15-s interstimulus interval (ISI). These animals were then divided into four groups of eight subjects with each group balanced for the mean response to the five tones. This matching procedure was employed to minimize between-group variability in startle amplitude (Davis and Wagner 1969). All behavioral training and testing took place during the dark part of the light-dark cycle, when startle amplitude is most constant (Davis and Sollberger 1971).

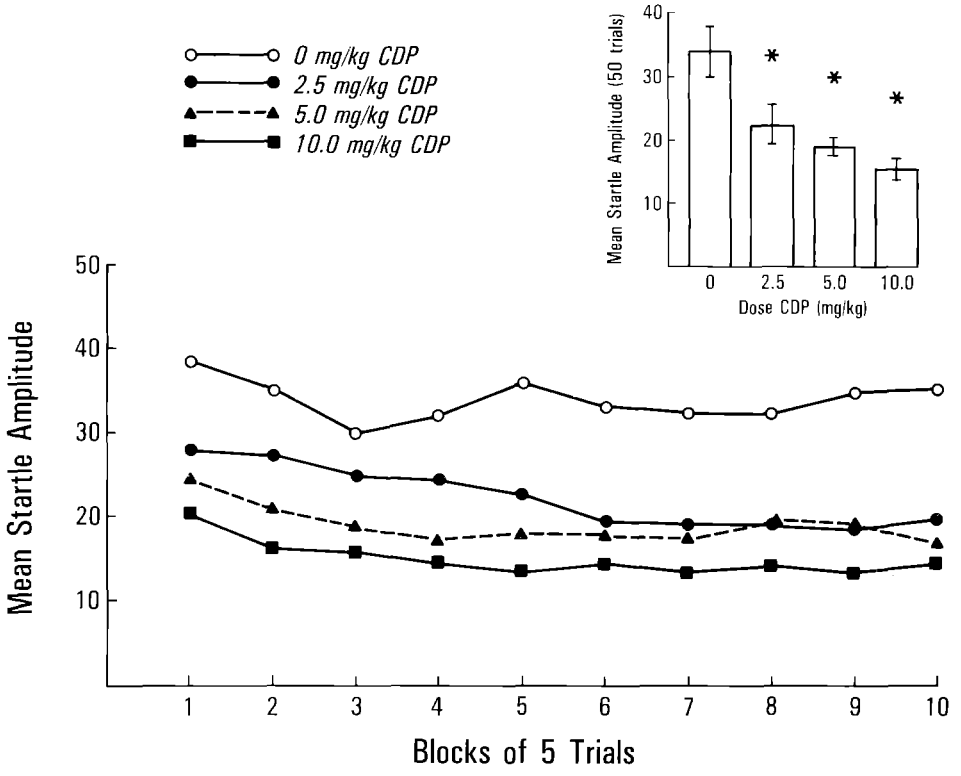

Fig. 2. Acoustic startle amplitude in rats following ICV infusion of 1 μg CRF and IP injection of chlordiazepoxide (0, 2.5, 5.0 or 10.0 mg/kg). Insert histogram indicates mean startle amplitude over 50 trials. *$P<0.05$, Newman-Keuls individual means comparison

On the day of testing, animals from each of the above groups received ICV infusion of one of four doses of rat CRF. Infusion was accomplished by replacing the stylet wire with an 8 mm stainless steel injector attached to a 1-m length of PE 10 tubing filled with infusate or saline vehicle. The tubing was then raised above the animal's head until flow began and 2 μl were infused over a 30–60-s period. Following infusion, the stylet wire was replaced and the animal was placed immediately into the stabilimeter cage. Five minutes later, the animal received 50 tones with a variable ISI that averaged 15 s.

Results and discussion

The results of Experiment I are shown in Fig. 1. Results analyzed using a two-way ANOVA with repeated measures on time revealed a significant effect of CRF ($F=5.11$; $df=3,31$; $P<0.01$); subsequent individual means comparison revealed that startle amplitudes in rats treated with 1.0 μg CRF were significantly ($P<0.001$, Newman-Keuls comparison) greater than those measured in saline-treated rats. Interestingly, startle amplitudes in animals treated with 10.0 μg CRF were not significantly elevated compared to saline-treated rats ($P>0.05$, Newman-Keuls comparison). All treatment groups showed significant habituation of the startle response over time ($F=16.47$; $df=9,288$; $P<0.001$), with no significant treatment × time interaxtion ($F=1.27$; $df=27,288$; NS). Thus, 1 μg CRF significantly enhanced startle amplitude, but did not significantly alter habituation.

These results are consistent with the notion that CRF has anxiogenic or fear-inducing properties in the rat, since CRF, like fear, potentiates ASR amplitude. The "inverted-U" dose-response properties of CRF are particularly interesting, since Davis and Astrachan (1978) have noted similar "non-monotonic" changes in fear-enhanced startle as a function of increasing shock intensity used in training. It is possible, however, that CRF might potentiate the ASR through mechanisms unrelated to fear or anxiety. The following experiments were designed to evaluate the contribution of "anxiogenic" properties of CRF to CRF-enhanced startle.

Experiment II

If CRF potentiates startle amplitude through its anxiogenic or fear-inducing properties, then drugs that reduce anxiety and decrease fear-enhanced startle should oppose CRF-potentiated startle. This prediction was tested by administering the anxiolytic benzodiazepine chlordiazepoxide (0, 2.5, 5.0 and 10.0 mg/kg IP) to rats 30 min prior to treatment with a dose of CRF (1 μg ICV) shown in Experiment I to maximally potentiate ASR amplitude.

Materials and methods

Procedure. Thirty-six animals were implanted with ICV cannulae and matched into four groups of nine animals following procedures described above. On the testing day, animals from each of the four groups were pretreated with one of four doses of chlordiazepoxide HCl in a saline vehicle (0, 2.5, 5.0 and 10.0 mg/kg) and returned to their home cage for 30 min. Each animal was then treated with 1 μg CRF, placed in the stabilimeter cage, and then presented with 50 tones using parameters described above.

Results and discussion

The results of Experiment II are seen in Fig. 2. A two-way ANOVA with repeated measures on time revealed a significant effect of CDP ($F=7.24$; $df=3,35$; $P<0.001$). Subse-

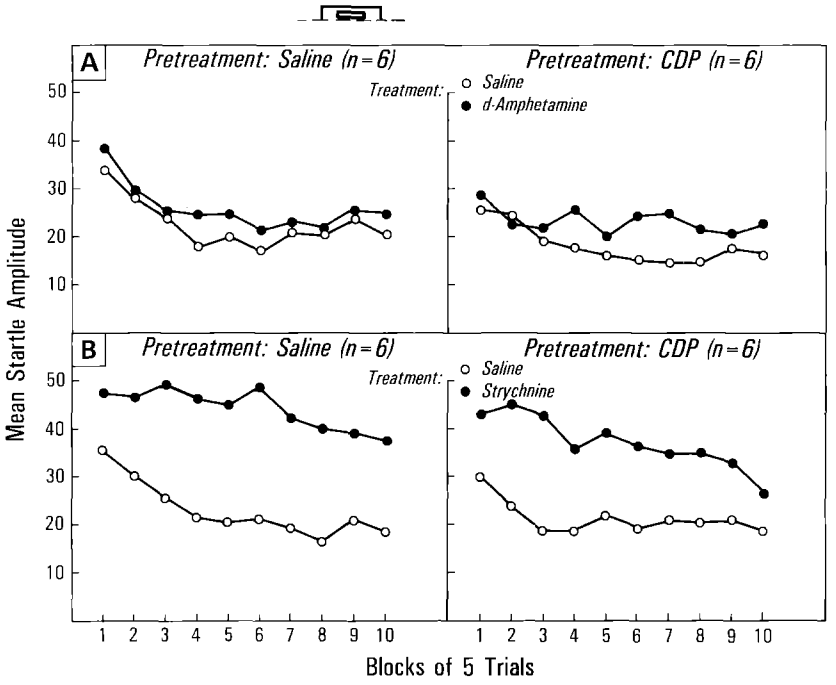

Fig. 3. Acoustic startle response in rats following pretreatment with saline or CDP (2.5 mg/kg IP). **A** Rats were treated with either saline or *d*-amphetamine (2.5 mg/kg SC). **B** Rats were treated with either saline or strychnine (0.75 mg/kg IP)

quent individual mean comparison with a Newman-Keuls test revealed that CDP significantly decreased ASR amplitude in CRF-treated rats at the lowest dose (2.5 mg/kg) of CDP tested ($P<0.05$). Startle habituation was evident in all CDP groups, as indicated by a significant effect of time ($F=5.04$; $df=9,324$; $P<0.001$), but no significant time × CDP interaction ($F<1$; $df=27,324$; NS). These results indicate that the anxiolytic chlordiazepoxide decreases ASR amplitude in CRF-treated animals. Startle is apparently at least as sensitive to the "anti-CRF" properties of CDP as is the Geller-Seifter conflict paradigm, in which 5.0 mg/kg CDP has been shown to reverse the effects of CRF on punished responding (Britton et al. 1985).

Experiment III

While the ability of CDP to decrease startle in CRF-treated rats might reflect its "anxiolytic" properties, it is also possible that these effects might result from a non-specific depressant action of CDP. If CDP produces a "non-specific" depression of ASR amplitude, then it would be predicted that CDP might lower baseline startle amplitude, or that it might oppose the startle-enhancing properties of drugs thought to potentiate startle through their action independent of anxiolytic properties. This prediction was tested by administering a dose of CDP effective in lowering ASR amplitude in Experiment II (2.5 mg/kg) to two groups of animals prior to treatment with either *d*-amphetamine (2.5 mg/kg SC) or strychnine (0.75 mg/kg IP).

Materials and methods

Twenty-four animals were randomly divided for testing with either *d*-amphetamine ($n=12$) or strychnine ($n=12$). In each case, they were matched into two groups of six animals using matching procedures described above. Four days later, animals tested with amphetamine were treated as follows: rats were pretreated with either saline (1 ml/kg IP, $n=6$) or CDP (2.5 mg/kg IP, $n=6$) and returned to their cages. Thirty minutes later half of the saline- and CDP-pretreated animals received treatments of saline (1 ml/kg, SC) and the other half of the animals received treatments of *d*-amphetamine (2.5 mg/kg). The animals were placed individually into the stabilimeter cage for 5 min, and then presented with 50 startle tones, using parameters identical to those of Experiments I and II. Five days later, this testing procedure was repeated, except that animals that had received saline treatment during the first test now received amphetamine, and vice versa. In this manner, each animal served as its own control, with treatment (saline versus amphetamine) forming a within-subject factor, and pretreatment (saline versus CDP) forming a between-subject factor. A separate group of 12 animals was tested in an identical fashion, except strychnine (0.75 mg/kg IP) was used instead of amphetamine. This experimental design has been used to demonstrate neuroleptic-induced blockade of amphetamine-potentiated startle (Kehne and Sorenson 1978). These dose of amphetamine (Davis et al. 1975) and strychnine (Kehne et al. 1981) were chosen in order to test the effects of CDP on a minimally-(amphetamine) and maximally-(strychnine) enhanced startle amplitude.

Results and discussion

The effects of CDP pretreatment on amphetamine-potentiated startle are seen in Fig. 3A. Analysis of variance revealed a significant effect of amphetamine treatment ($F=11.99$; $df=1,228$; $P<0.01$), but not of CDP pretreatment ($F<1$; $df=1,11$; NS) and no amphetamine × CDP interaction ($F<1$; $df=1,228$; NS). There was a significant effect of time ($F=8.86$; $df=9,90$; $P<0.001$), but no time × treatment ($F=1.30$; $df=9,90$; NS) or time × treatment × pretreatment interaction ($F<1$; $df=9,90$; NS).

The effects of CDP pretreatment on strychnine-potentiated startle are seen in Fig. 3B. Analysis of variance revealed a significant effect of strychnine treatment ($F=15.89$; $df=1,228$; $P<0.005$), but not CDP pretreatment ($F<1$; $df=1,11$; NS) and no strychnine × CDP interaction

($F<1$; $df=1,228$; NS). There was a significant effect of time ($F=9.75$; $df=9,90$; $P<0.001$), but no time × treatment ($F=1.66$; $df=9,90$; NS) or time × treatment × pretreatment interactions ($F<1$; $df=9,90$; NS). Thus, CDP did not significantly modify baseline startle in two separate between-group comparisons, nor did it significantly decrease either amphetamine- or strychnine-potentiated startle amplitude. These results do not support the hypothesis that CDP might decrease CRF-potentiated startle through "non-specific" depressant effects, since no evidence of such depressant properties of CDP was detected in any of the two between-group or two within-group comparisons in this experiment.

General discussion

The present findings add to a growing body of evidence that exogenously administered CRF produces behavioral changes in the rat that mimic changes normally exhibited during stressful or anxiogenic situations (Britton et al. 1982; Sutton et al. 1982; Britton et al. 1985). In the present study, CRF was found to potentiate the amplitude of the acoustic startle response; this CRF effect was decreased in a dose-dependent manner by the anxiolytic chlordiazepoxide. In a separate test, chlordiazepoxide did not depress baseline startle amplitude, nor did it antagonize amphetamine- or strychnine-potentiated startle. Thus, CRF produced "fear-like" increases in the ASR that were selectively reversed by a drug known to decrease anxiety.

While the present study tested the effects of exogenous CRF on ASR amplitude, it is tempting to speculate that endogenous CRF might act within the CNS to produce neural changes responsible for fear-potentiated startle. This notion is supported by several independent findings. First, stressors that potentiate startle amplitude (e.g., footshock) (Davis and Astrachan 1978) cause the release of CRF into the cerebrospinal fluid (Britton et al. 1984). Second, low intensity electrical stimulation of the central nucleus of the amygdala – the region with the highest concentration of CRF outside the hypothalamus (Swanson et al. 1983) – produces robust increases in acoustic startle amplitude (M. Davis, personal communication). Third, of all brain regions studied to date, only lesions of the central nucleus of the amygdala selectively block fear-potentiated startle (Mondlock and Davis 1985). Finally, doses of CRF that produce evidence of "anxiety" in other behavioral tests (Britton et al. 1985) increase startle amplitude (present study), and these effects of CRF, like those of conditioned fear (Davis 1979a), are blocked selectively by anxiolytic drugs (present study).

Numerous studies have documented the usefulness of the acoustic startle response as a sensitive model for studying the effects of drugs and environmental stimuli on states of arousal, fear or anxiety (Prosser and Hunter 1936; Moyer and Bunnell 1960b; Wager 1963; Armus et al. 1964; Chi 1965; Korn and Moyer 1965; Hoffman and Stitt 1969; Mellgren 1969; Davis and Astrachan 1978; Davis 1979a, b). In the present study, we have demonstrated that an endogenous neuropeptide that serves to mobilize the normal hypothalamic-pituitary-adrenal axis response to stress (Vale et al.1981) also stimulates "anxiogenic" changes in the acoustic startle response that are selectively opposed by the clinically-effective anxiolytic chlordiazepoxide. These results suggests that endogenous CRF might normally serve to potentiate acoustic startle as well as other behavioral responses during states of enhanced fear or anxiety.

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