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Characterization of the effects of corticotropin-releasing factor on prepulse inhibition of the acoustic startle response in Brown Norway and Wistar-Kyoto rats

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Abstract

Sensori-motor gating, as assessed by prepulse inhibition of the startle response is diminished in patients with schizophrenia. We have previously shown that inbred Brown Norway (BN) rats display significantly less prepulse inhibition of the acoustic startle response than inbred Wistar-Kyoto (WKY) rats, and that prepulse inhibition is decreased by central administration of the neuropeptide, corticotropin-releasing factor (CRF) in both strains. The present study was conducted to establish whether peripheral administration of CRF alters prepulse inhibition, whether a low, threshold dose for decreasing prepulse inhibition is the same in the two rat strains, and whether central administration of a CRF receptor antagonist enhances prepulse inhibition in the BN strain. CRF-induced behavioral activation was also examined to determine whether the two rat strains are differentially sensitive to a behavioral effect of CRF that does not involve the startle response. In each experiment, BN rats showed significantly less prepulse inhibition than WKY rats. Subcutaneous administration of CRF had no affect on startle amplitude or prepulse inhibition of the startle response in either rat strain. In BN, but not in WKY rats, low-dose CRF (0.3 μg) decreased prepulse inhibition. However, doses of CRF that did not alter prepulse inhibition in the WKY strain, did result in behavioral activation. No dose of CRF tested affected baseline startle amplitude. Central administration of the CRF receptor antagonist, astressin had no effect on prepulse inhibition or startle amplitude in either rat strain. Central administration of the CRF receptor antagonist, D-Phe CRF $_{12–41}$ had no effect on prepulse inhibition in WKY rats, resulted in a only a small, non-significant increase in prepulse inhibition in BN rats, while it decreased startle amplitude. The results suggest that CRF reduces prepulse inhibition of the acoustic startle response independently of effects on the pituitary-adrenal axis, and that endogenous CRF has at most, a minor role in the low prepulse inhibition found in BN rats.

Keywords: Brown Norway rat; Schizophrenia; Sensorimotor gating; Startle amplitude; Wistar-Kyoto rat

1. Introduction

Presentation of a non-startling stimulus, shortly prior to presentation of a startling stimulus, results in inhibition of the startle response (Hoffman and Searle, 1965), a phenomenon known as prepulse inhibition. Prepulse inhibition is measure of sensori-motor gating that is thought to occur so that processing of the prepulse stimulus is not disrupted by a large-amplitude startle response, and deficits in prepulse inhibition might reflect a deficit in the ability to integrate information (Braff and Geyer, 1990). Prepulse inhibition is diminished in patients with a number of psychiatric disorders including schizophrenia (Braff et al., 1978; Geyer et al., 1990; Mackeprang et al., 2002; Parwani et al., 2000), as well as in the unaffected relatives of patients with schizophrenia (Cadenhead et al., 2000), suggesting a genetic influence. Indeed in persons with no psychiatric diagnosis, prepulse inhibition is highly heritable (Anokhin et al., 2003).

In rodents, prepulse inhibition of the acoustic startle response can be achieved using parameters that are nearly identical to those employed in studies with human subjects,

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making the paradigm a useful tool for studying the roles of specific neurotransmitters and genetic influences in sensorimotor gating deficits. Drugs that increase extracellular concentrations of dopamine, or are dopamine receptor agonists, disrupt prepulse inhibition in rats (Geyer et al., 1990; Mansbach et al., 1988). Serotonin (5-HT) releasers, and direct agonists at 5-HT_{1B} or 5-HT_2 receptors, also decrease prepulse inhibition in rodents (Martinez and Geyer, 1997; also see Swerdlow and Geyer, 1998 for review). In the absence of pharmacological manipulations, there are differences in prepulse inhibition among rat strains (Faraday et al., 1999; Hall et al., 1997; Varty and Geyer, 1998). Additionally, there are rat strain differences in sensitivity to the disruptive effects of dopamine receptor agonists on prepulse inhibition (Swerdlow et al., 2000; Varty and Geyer, 1998).

We have shown that inbred Brown Norway (BN) rats show significantly less prepulse inhibition than inbred Wistar-Kyoto (WKY) rats (Palmer et al., 2000; Conti et al., 2002), and that central administration of a relatively high dose of corticotropin-releasing factor (CRF) diminishes prepulse inhibition in both the BN and WKY strains. While CRF is a hypothalamic peptide that is released during stress to result in adrenocorticotropic hormone (ACTH) release, (Rivier and Vale, 1983), CRF from non-hypothalamic sources acts as a neurotransmitter during stress (Gabr et al., 1991; Van Bockstaele et al., 1998). CRF-immunoreactive cells and fibers are distributed in many regions of the brain, including the central nucleus of the amygdala, the hippocampus, the cortex, and the dorsal raphe nucleus (Kirby et al., 2000; Lahmame et al., 1997; Swanson et al., 1983). CRF receptors are expressed in cortex, striatum, hippocampus, the nucleus accumbens, and the basolateral nucleus of the amygdala (Chalmers et al., 1995; De Souza, 1987; Radulovic et al., 1998), areas shown to be important for regulation of prepulse inhibition (Bakshi and Geyer, 1999; Swerdlow et al., 1994).

The reason for the low basal prepulse inhibition in the BN rat strain is not known. One possibility is that BN rats show low levels of prepulse inhibition because they have higher levels of endogenous CRF in cortex than WKY rats (Lahmame et al., 1997). Indeed, transgenic mice which overexpress CRF show diminished prepulse inhibition compared to wild-type controls (Dirks et al., 2002). If high endogenous levels of CRF result in the relatively poor prepulse inhibition in the BN strain, then central administration of a CRF receptor antagonist might improve prepulse inhibition in this strain.

The present studies were designed to further investigate the role of CRF on prepulse inhibition in the WKY and BN rat strains by asking three questions: (1) Dose peripheral administration of CRF, at doses that result in activation of the hypothalamic–pituitary–adrenal axis (Cador et al., 1992), also decrease prepulse inhibition?; (2) Is there a differential effect of CRF on prepulse inhibition in WKY and BN rats at doses of CRF that are considerably lower than those previously used?; (3) Does central administration of a CRF receptor antagonist enhance the low levels of prepulse inhibition seen in the BN rat strain?

2. Materials and methods

2.1. Animals

Experiment 1 was performed while the author was at the University of California, San Diego (UCSD), and experiments 2 and 3 were performed at the University of Connecticut Health Center (UCHC). Rats for all experiments were males, weighing 250–275 g at the start of the experiment. WKY rats were obtained from Charles River, and Brown Norway rats were obtained from Harlan, Sprague Dawley. Rats were allowed to acclimate to our vivarium for 1 week prior to the start of any experimental procedure. The vivaria at both UCSD and UCHC are maintained on a 12 h light/dark cycle with laboratory chow and water available ad libitum. All rats were housed 2–3/cage until undergoing surgery to have intracerebroventricular (i.c.v.) cannula implanted (experiments 2 and 3). Thereafter, the rats were single-housed for 5–7 days until testing. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Surgery to implant i.c.v. guide cannula

In experiments 2 and 3, rats were anesthetized with a mixture of isoflurane-in-air (1.5%) and placed into a stereotaxic instrument equipped with blunt ear bars. A stainless-steel guide cannula (22 gauge) was aimed at the lateral ventricle (1.0 mm posterior and 2.0 mm lateral to Bregma) for subsequent i.c.v. infusion of either saline, hCRF (experiment 2), or one of two CRF receptor antagonists (experiment 3). Two jewelers' screws were placed into the skull, and the entire assembly was held in place with dental cement. Testing occurred following a 5–7 day recovery period.

2.3. CRF and CRF receptor antagonists

Both hCRF, and the CRF receptor antagonists, cyclo(30–33)D-Phe^{12},$Nle^{21,38}$,Glu^{30},Lys^{33}r$CRF_{-(12-41)}$ (astressin), and D-Phe^{12}, $Nle^{21,38}$,$C^{\alpha}Me$ Leu^{37}r$CRF_{(12-41)}$ (D-Phe) were kindly provided by Dr. Jean Rivier (Salk Institute, La Jolla, CA).

2.4. CRF and CRF receptor antagonist administration and observation of active behaviors

In experiment 1, rats received a subcutaneous (s.c.) injection of either saline or CRF (1.0 or 3.0 μg; 1.0 ml/kg volume) 30 min prior to testing. For i.c.v. infusions in experiments 2 and 3, rats were gently held while an infusion

cannula (28 gauge) attached to PE 20 tubing, was lowered into the guide. A Hamilton syringe was used to deliver saline, CRF, or a CRF receptor antagonist through the infusion cannula over a 1 min period. The infusion cannula remained in place for an additional 30 s following the infusion. In experiment 2, rats received an i.c.v. infusion of either saline or one dose of CRF (0.1–1.0 μg) in 6.0 μl vol. Immediately following the i.c.v. infusion, rats were placed back into the home cage, and 15 min later, were observed for all active behaviors over a 15 min period. The behaviors included grooming, burrowing in bedding, rearing, walking, chewing, or head movements. Rats in this experiment were also re-tested 72 h after the first test, following re-administration of the same infusate they had received prior to the first test (n=7–11 rats/group). This was done in order to examine whether the effect of CRF on prepulse inhibition is unchanged, enhanced, or attenuated with repeated administration and testing. In experiment 3, rats received an i.c.v. infusion of either saline, the CRF receptor antagonist, astressin (5.0 or 10.0 μg), or the CRF receptor antagonist, D-Phe (5.0 or 10.0 μg) 10 min prior to testing. All testing took place between the hours of 10:00 am and 3:00 pm.

2.5. Apparatus

For testing, rats were placed into a clear acrylic cylindrical chamber (10 cm diameter; 14 cm length) that is enclosed by a sound- and vibration-attenuating cabinet equipped with a 20 W incandescent bulb and a fan for ventilation (San Diego Instruments). The chamber sits upon a base, under which is a piezoelectric accelerometer which detects whole body startle responses. Output signals from the accelerometer are collected as 100 sequential 1 ms measurements starting at the onset of the startle stimulus. The signals are rectified, digitized, and stored on computer by an SR-LAB program (San Diego Instruments). Chambers are calibrated each day, and matched for sound intensity. Delivery of white noise acoustic stimuli, through a horn tweeter (Radio Shack), is also controlled by the SR-LAB program.

2.6. Testing for prepulse inhibition and acoustic startle response

Testing was conducted as previously described (Conti et al., 2002). Rats were placed into a startle chamber for a 5 min acclimation period prior to the delivery of any stimulus. All stimuli were presented on a 70 dB background. In all experiments, the first and last six trials of the session were acoustic startle trials in which a 120 dB, white noise burst was presented for 40 ms. In experiment 1, the middle 50 trials consisted of five stimulus types presented in a pseudo-random order: Acoustic startle stimuli in the absence of a prepulse stimulus (12 trials); Prepulse stimuli, 3, 6 or 12 dB (20 ms) above background, preceding the startle stimulus by 100 ms (10 trials of each prepulse intensity); No stimulus (8 trials). In experiments 2 and 3, five intensities of prepulse stimuli were used: 3, 6, 12, 15, or 18 dB (20 ms) above background, preceding the startle stimulus by 100 ms (10 trials of each prepulse intensity). These additional prepulse stimulus intensities were added in order to ensure adequate prepulse inhibition in the BN strain which is sometimes not found with the lowest prepulse stimulus intensities. The inter-trial interval averaged 15 s. Average startle amplitude during the 100 ms following the onset of each startle stimulus was recorded and stored on a computer.

2.7. Data analysis

Percent prepulse inhibition was calculated for each rat at each prepulse stimulus intensity as follows:

$$\%\ \text{Prepulse Inhibition} = 100 - (100 \times [\text{prepulse}/\text{startle}]),$$

where *prepulse* is the average startle amplitude on trials in which there was a prepulse stimulus, and *startle* is the average amplitude on the trials in which the startle stimulus was presented alone. Data from the first and last 6 trials were not used in this calculation. Average startle amplitude over the 24 trials on which the startling stimulus was presented alone was also calculated. In order to examine whether CRF altered habituation of the startle response, percent habituation was calculated as: 100(average of first 6 trials−average of last 6 trials)/average of last 6 trials.

For the percent prepulse inhibition data, a 3-way analysis of variance (ANOVA) was used, with rat strain and CRF dose as between-subjects factors and prepulse stimulus intensity as a repeated measure. The percent habituation data, and the time spent engaged in active behaviors, were each subjected to a 2-way ANOVA with rat strain and CRF dose as between subjects factors. For experiment 3, separate 2-way ANOVAs with rat strain and CRF receptor antagonist dose as between-subjects factors, were used to examine the effects of astressin and D-Phe. Since the two CRF receptor antagonists were tested in the same experiment, only one saline control group was used for comparison to both. When significant main effects were found, Tukey post-hoc tests were employed to examine differences between pairs of doses.

3. Results

3.1. Experiment 1

The effects of s.c. administration of CRF on percent prepulse inhibition in the two rat strains is shown in Fig. 1A. There was a significant effect of rat strain, $F(1,54)=18.1$, $P<0.001$. There was no effect of CRF dose, $P>0.05$, and no rat strain×CRF dose interaction, $P>0.05$. Startle amplitude following s.c. administration of CRF is shown in Fig. 1B. There was a significant effect of rat strain, $F(1,54)=5.8$,

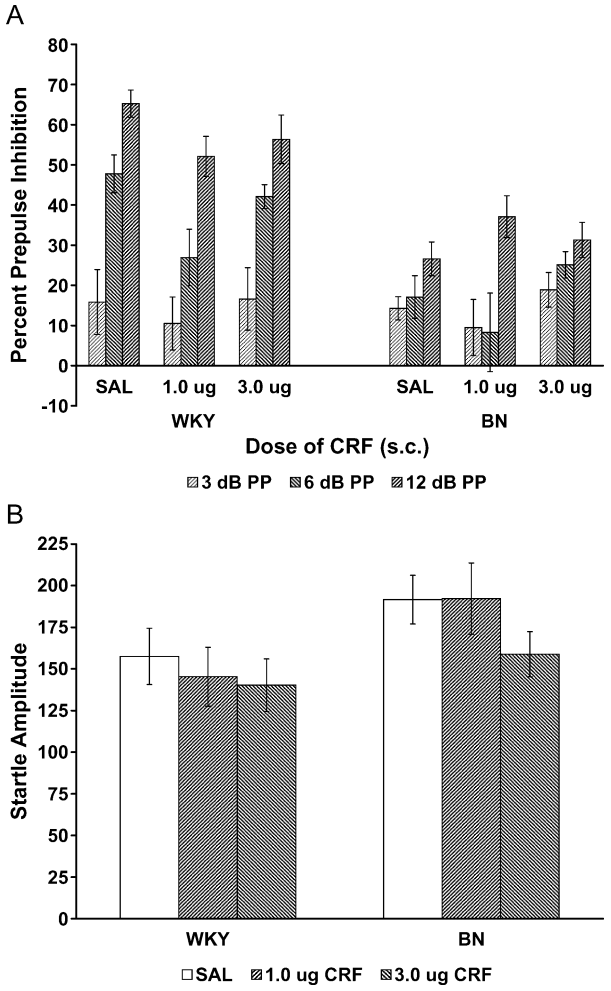

Fig. 1. The effect of CRF (s.c.) on percent prepulse inhibition (A) and startle amplitude (B) in WKY and BN rats. Shown are the group means (±SEM). There was a significant effect of rat strain on percent prepulse inhibition, but no effect of CRF dose, and no rat strain×CRF dose interaction following s.c. administration of CRF (A). There was also a significant effect of rat strain on startle amplitude (B), but no effect of CRF dose, and no interaction (see text).

$P<0.02$, but no effect of CRF dose, $F(2,54)=1.2$, $P>0.05$. Percent habituation of startle amplitude is shown in Table 1. There was a significant effect of rat strain, $F(1,54)=20.9$, $P<0.001$, but no effect of CRF dose, ($P>0.05$), and no rat strain×CRF dose interaction, ($P>0.05$).

3.2. Experiment 2

The effect of CRF (i.c.v.) on percent prepulse inhibition in WKY and BN rats is shown in Fig. 2. The results on the first of the two tests (Test 1) are shown in 2A (WKY) and 2B (BN). The results of Test 2, which took place 72 h after Test 1, are shown in 2C (WKY) and 2D (BN). On Test 1, there was a significant effect of rat strain, $F(1,59)$,=7.0, $P=0.01$, and a significant effect of CRF dose $F(3,59)=3.8$, $P<0.02$. There was no significant rat strain×CRF dose interaction. There was also a significant effect of prepulse stimulus intensity, $F(4,236)=88.0$, $P<0.001$, and a prepulse intensity×rat strain interaction, $F(4,236)=12.4$, $P<0.001$. In WKY rats alone, there was no significant effect of CRF dose, $P>0.05$. In BN rats, there was an overall significant effect of CRF dose, $F(3,30)=3.8$, $P<0.02$. Tukey post hoc tests on the data from the BN strain, revealed that there was a significant difference between saline and 0.3 μg CRF ($P<0.05$), as well as between 0.1 μg and 0.3 μg CRF ($P<0.05$). When rats were tested for prepulse inhibition a second time (Test 2: Fig. 2C and D), under the same dose as was given in Test 1, there was again a significant effect of rat strain, $F(1,52)=31.1$, $P<0.001$. On this test, there was also a significant overall effect of CRF dose, $F(3,52)=2.9$, $P<0.05$, and a rat strain×CRF dose interaction, $F(3,52)=4.35$, $P<0.01$. Again on Test 2, there was no significant effect of CRF dose in the WKY rats ($P\geqslant 0.05$). There was a significant effect of CRF dose in the BN rats, $F(3,30)=8.6$, $P<0.001$. Tukey tests revealed that again on Test 2, there was a significant difference between the effects of saline and the 0.3 μg dose of CRF, ($P<0.001$), as well as between the effects of 0.1 μg and 0.3 μg dose of CRF, ($P=0.005$). Additionally, on Test 2, there was a significant difference between the effects of the 0.3 μg and 1.0 μg dose of CRF in the BN rats ($P=0.001$). A separate ANOVA to examine the effect of Test (Test 1 vs. Test 2) was conducted using the average percent prepulse inhibition across all prepulse stimulus intensities. It revealed a significant effect of rat strain, $F(1,48)=17.7$, $P<0.001$, a significant effect of CRF dose, $F(3,48)=3.3$, $P<0.05$, and a marginal rat strain×dose interaction, $F(3,48)=2.7$, $P=0.054$. There was also a significant effect of Test, $F(1,48)=19.0$, $P<0.001$, and

Table 1
Percent habituation of the startle response (mean±SEM) from the first block of 6 trials to the last block of 6 trails in each of the three experiments

Experiment 1		
Dose of CRF (s.c.)	WKY	BN
SAL	383.2±82.7	74.0±15.6
1.0 μg	383.5±105.6	159.4±36.4
3.0 μg	346.3±84.3	121.1±29.8
Experiment 2		
Dose of CRF (i.c.v)	WKY	BN
Test 1		
SAL	335.2±108.5	92.1±35.1
0.1 μg	238.1±80.7	151.3±71.5
0.3 μg	216.1±61.1	167.4±54.9
1.0 μg	318.2±78.4	164.3±51.4
Test 2		
SAL	154.7±48.6	77.0±31.8
0.1 μg	236.4±8.1	112.8±33.5
0.3 μg	146.2±71.7	103.4±48.1
1.0 μg	216.9±85.7	160.9±52.0

In Experiment 1, and on Day 1 of Experiment 2 each experiment, there was a significant effect of rat strain, with WKY rats showing greater percent habituation than BN rats. Neither s.c., nor i.c.v. CRF had a significant effect on percent habituation, and in no experiment was there a rat strain×CRF dose interaction (see text for details).

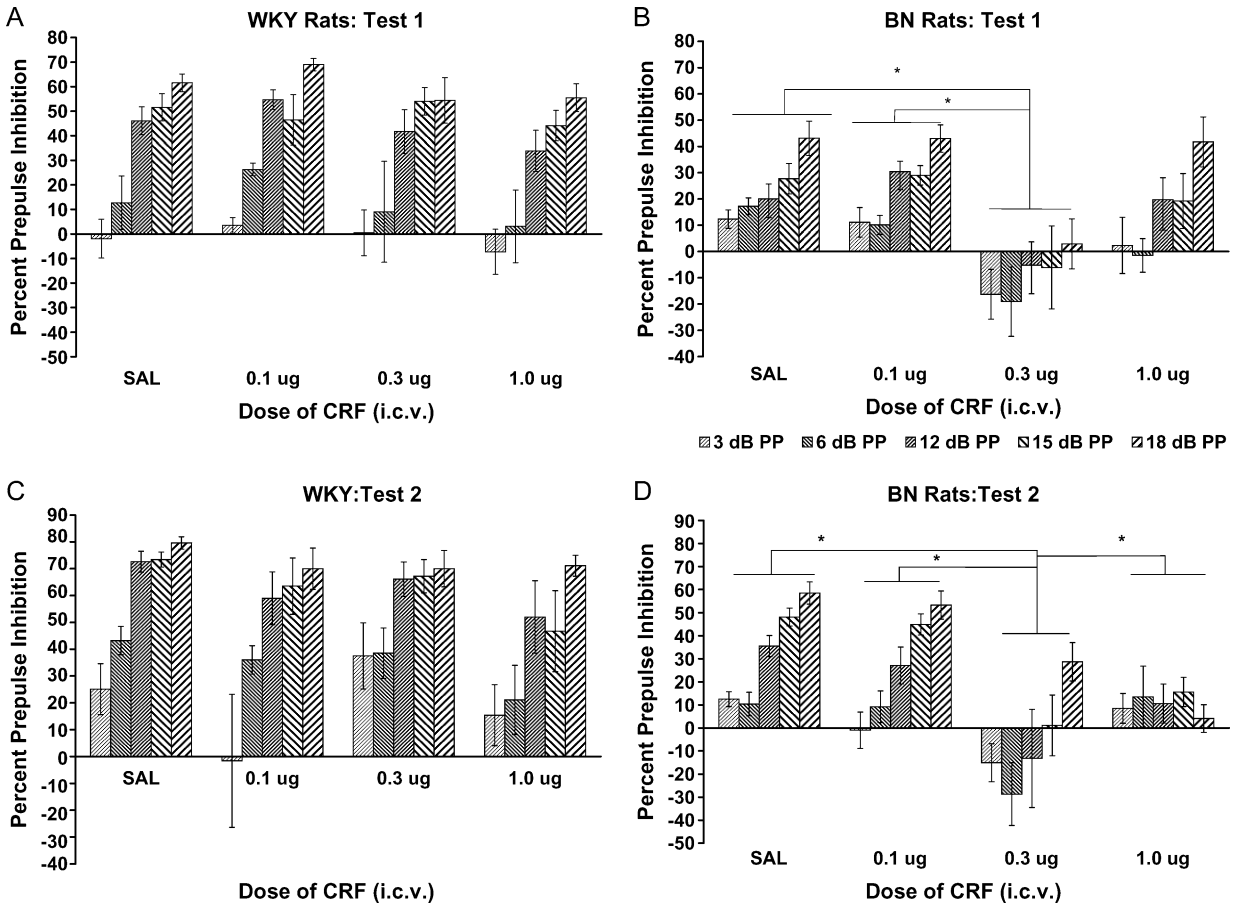

Fig. 2. The effect of CRF (i.c.v.) on percent prepulse inhibition in WKY and BN. Shown are mean (±SEM) on Test 1 (A and B) and Test 2 (C and D) which took place 72 h later. Rats received the same infusate on both tests. On Test 1, there was a significant effect of rat strain, and a significant rat strain×CRF dose interaction (see text). In WKY rats (A) there was no significant effect of any dose of CRF on percent prepulse inhibition, although the 1.0 μg dose tended to diminish prepulse inhibition. In the BN rats (B), there was significantly less prepulse inhibition following the 0.3 μg dose of CRF than following either saline, or the 0.1 μg dose of CRF (*). On Test 2, the 0.3 μg dose of CRF significantly reduced percent prepulse inhibition compared to each of the other 3 groups (*) in BN rats (see text for details).

a significant rat strain×Test interaction, $F(1, 48)=6.0$, $P<0.02$.

Fig. 3 shows the effect of CRF in on startle amplitude in both rat strains on Test 1 (3A and 3B) and Test 2 (3C and 3D). On Test 1, there was no effect of rat strain ($P>0.05$), no effect of CRF dose ($P>0.05$), and no rat strain×dose interaction ($P>0.05$). There was a significant effect of trial block, $F(3,177)=77.8$, $P<0.001$, and a significant rat strain×trial block interaction, $F(3,177)=5.8$, $P=0.001$. This interaction reflects the finding that WKY rats displayed significantly greater percent habituation of the startle response than BN rats, $F(1,59)=6.5$, $P<0.02$ (see Table 1). In order to examine whether the overall lack of effect of CRF on startle amplitude was due to the introduction of prepulse stimuli to the session, the effects of rat strain and CRF dose on the first trial block alone were examined. The ANOVA revealed no significant effect of rat strain ($P>0.05$), or CRF dose ($P>0.05$), and no interaction ($P>0.05$). On Test 2, there was no significant effect of rat strain ($P>0.05$), or of CRF dose ($P>0.05$) on startle amplitude, and there was no interaction ($P>0.05$) (Fig. 3B). There was a significant effect of trial block, $F(3,156)=46.3$, $P<0.001$, as well as a significant rat strain×trial block interaction, $F(3,156)=8.7$, $P<0.001$.

Time spent active during the 15 min observation period that preceded Test 1 (above) is shown in Fig. 4. There was a significant effect of rat strain, $F(1,55)=30.4$, $P<0.001$ and of CRF dose, $F(3,55)=13.9$, $P<0.001$, but no significant interaction.

3.3. Experiment 3

The effects of the CRF receptor antagonists, astressin and D-Phe, on prepulse inhibition in the two rat strains are shown in Fig. 5A and B. For astressin, there was a significant effect of rat strain, $F(1,41)=49.8$, $P<0.001$. There was no significant effect of astressin dose, $F(2,41)=1.4$, $P>0.05$, and there was a marginal rat strain×dose interaction, $F(2,41)=2.9$, $P=0.065$. There was also a significant effect of prepulse intensity, $F(4,164)=85.1$, $P<0.001$, as well as a significant rat strain×prepulse interaction, $F(4,164)=20.6$, $P<0.001$. For D-Phe, there

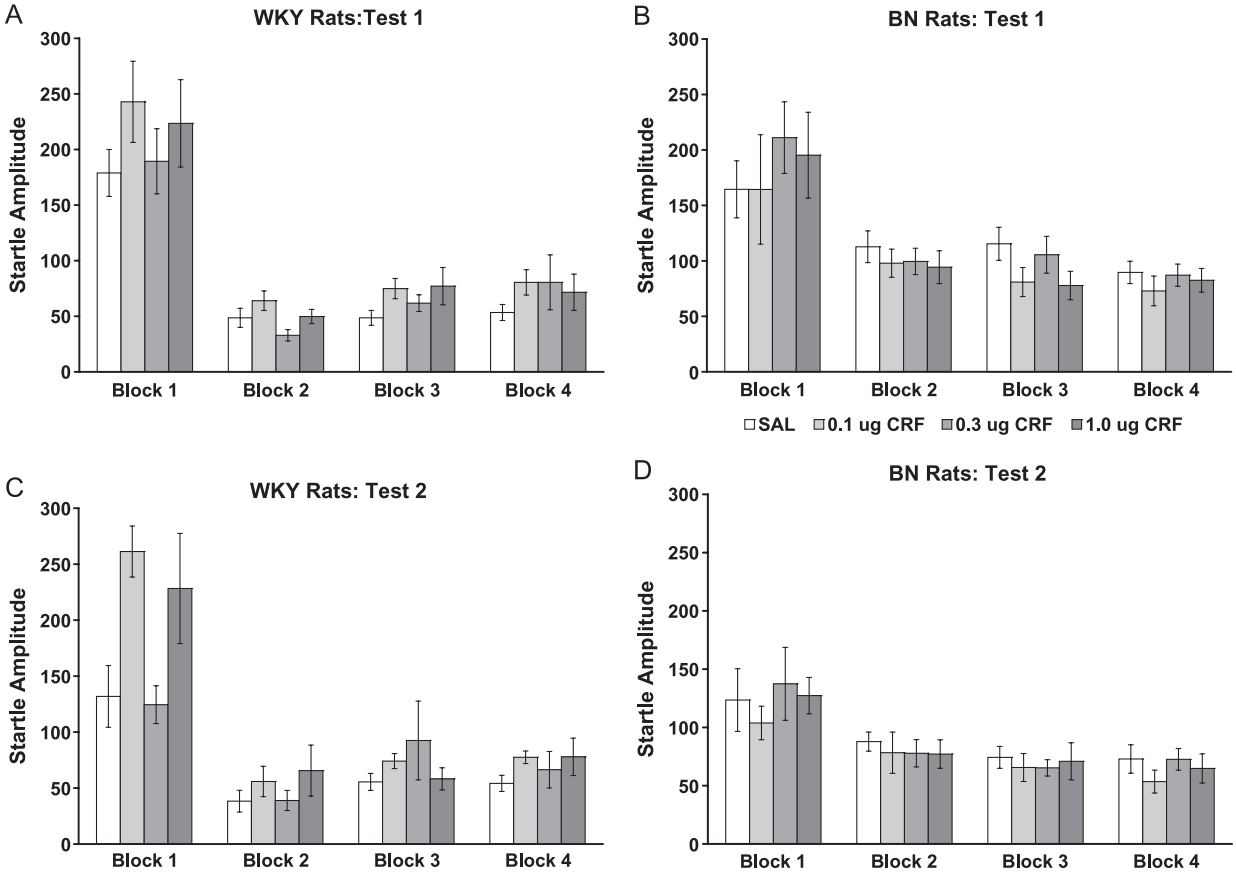

Fig. 3. The effect of CRF (i.c.v.) on startle amplitude on Test 1 (A) and Test 2 (B) of Experiment 2. On Test 1, there was a marginal effect of rat strain, and no effect of CRF dose. On Test 2, there was no effect of rat strain or CRF dose, and surprisingly, no CRF dose×trial block interaction.

was also a significant effect of rat strain, $F(1,45)=66.9$, $P<0.001$. There was no overall significant effect of D-Phe dose, $F(2,45)=1.4$, $P>0.05$, and no strain×dose interaction, $P>0.05$. Again, there was a significant effect of prepulse intensity, $F(4,180)=102.2$, $P<0.001$, and a strain×prepulse interaction, $F(4,180)=26.2$, $P<0.001$.

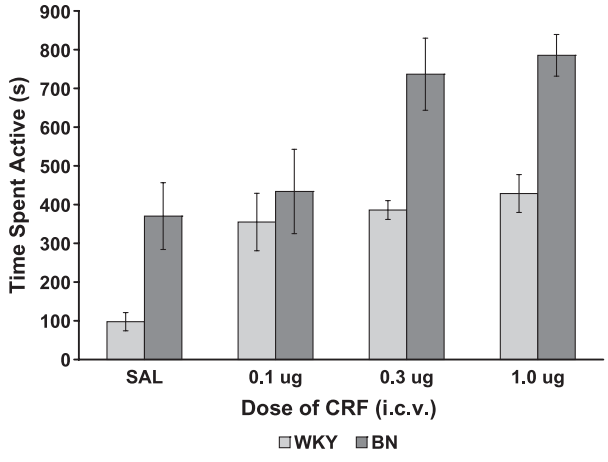

Fig. 4. Time spent engaged in active behaviors following CRF infusion. CRF increased time spent active in both strains.

The effects of the CRF antagonists on startle amplitude in the two rat strains are shown in Fig. 6. For astressin, there was no significant effect of dose, $F(2,41)=1.26$, $P>0.05$, or of rat strain, $F<1.0$, and no interaction between these two terms, $F<1.0$. For D-Phe, there was a significant effect of dose on startle amplitude, $F(2,45)=3.3$, $P<0.05$, but no significant effect of rat strain, $F(1,45)<1.0$.

4. Discussion

Subcutaneous administration of doses of CRF that cause the release of ACTH and corticosterone (Cador et al., 1992), did not alter prepulse inhibition in either rat strain in the present experiment, suggesting that the effect of i.c.v. CRF on prepulse inhibition is independent of its effects the hypothalamic–pituitary–adrenal axis. This result is in agreement with those showing that neither acute, nor repeated administration of corticosterone decreases prepulse inhibition in rats (Czyrak et al., 2003). In another report, adrenalectomized mice with corticosterone replacement showed less prepulse inhibition than those without replacement (Stevens et al., 2001), although this result could be interpreted to suggest that adrenalectomy decreases prepulse inhibition below control levels. The present result is also

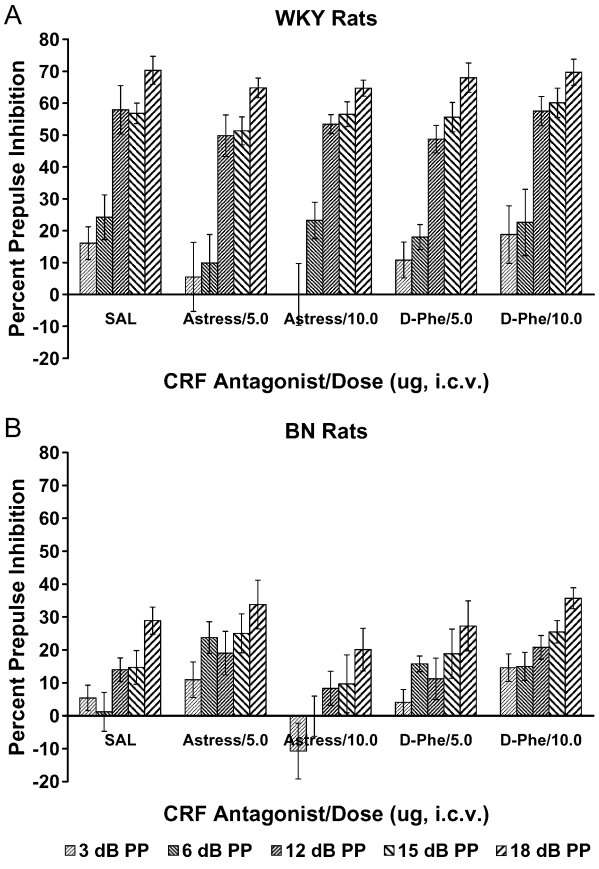

Fig. 5. The effect of the CRF receptor antagonists, astressin (A) and D-Phe (B), on prepulse inhibition in the WKY and BN strains. Neither astressin, nor D-Phe had a significant effect on percent prepulse inhibition.

consistent with a number of others showing that the behavioral effects of central administration of CRF do not depend on activation of the hypothalamic–pituitary–adrenal axis (Berridge and Dunn, 1989; Campbell et al., 2004; Eaves et al., 1985; Veldhuis and De Weid, 1984). Thus, although i.c.v. administration of CRF increases ACTH and corticosterone release (Song et al., 1995), and circulating corticosterone can affect the hippocampus and the amygdala (see McEwen, 1979; Makino et al., 2002 for review), the effect of i.c.v CRF on prepulse inhibition does not appear to be due to corticosterone.

The results of the present experiments also reveal that i.c.v. administration of a low dose of CRF (0.3 μg) diminishes prepulse inhibition in BN, but not in WKY rats, while a 3.0 μg dose of CRF has been shown to significantly diminish prepulse inhibition in both rat strains (Conti et al., 2002). Thus, the BN rats may be more sensitive to the effects of CRF on prepulse inhibition than the WKY rats. The 0.3 μg dose of CRF also decreased prepulse inhibition in the BN rats on Test 2. Additionally, CRF produced a marginally significant effect on in the WKY on Test 2. Thus, there was no tolerance to the effect of CRF on prepulse inhibition. Additionally, the difference in prepulse inhibition between the saline-treated WKY and BN rats was retained on the second test, suggesting that the decrease in prepulse inhibition in the BN rats is not a transient phenomenon, and that there will be a reliable difference between the two strains in paradigms in which repeated testing is desirable.

It is possible that CRF affects prepulse inhibition via multiple mechanisms in the BN rats, with some doses acting directly to affect prepulse inhibition, and others acting to recruit additional neurotransmitters that also affect prepulse inhibition. For example, low doses of CRF decrease 5-HT concentrations in the lateral septum, while higher doses of CRF increase 5-HT concentrations (Price et al., 1998). CRF also increases extracellular concentrations of dopamine and norepinephrine (Kalivas et al., 1987; Lavicky and Dunn, 1993; Matsuzaki et al., 1989; Page and Abercrombie, 1999), and may indirectly alter prepulse inhibition via affects on these catecholamines. Dirks et al. (2003) have shown that the deficit in prepulse inhibition seen in CRF over-expressing mice is attenuated by both typical and atypical antipsychotics, suggesting that the effects of CRF on prepulse inhibition might be mediated through the mono-amine systems that are the targets of these drugs.

CRF did not significantly increase startle amplitude at any dose in either rat strain in the present experiment. CRF over-expressing mice, which also display diminished prepulse inhibition, do not display enhanced startle compared to wild-type (WT) control mice (Dirks et al., 2002). Yet, exogenously administered CRF has been shown to increase startle amplitude at doses that are within the range employed in the present experiment (Jones et al., 1998; Liang et al., 1992; Swerdlow et al., 1989). In the present paradigm, CRF was administered 30 min prior to the start of testing, and the test session lasted for 25 min. In the experiment by Liang et al. (1992) the effect of a 1.0 μg dose of CRF on startle amplitude began 20–30 min after i.c.v. infusion and was increased with time thereafter. Thus, in the present experiments, CRF may have failed to affect startle

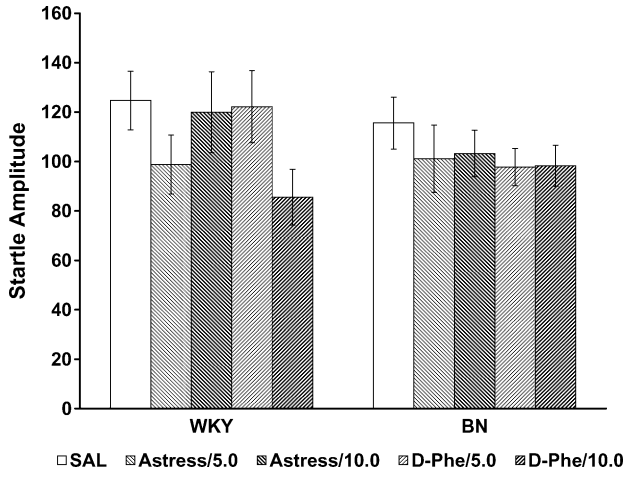

Fig. 6. The effects of the CRF receptor antagonists, astressin (A) and D-Phe (B), on startle amplitude in the WKY and BN strains. Astressin had no effect on startle amplitude in either rat strain. D-Phe decreased startle amplitude (see text for details).

amplitude because not enough time was allowed to elapse between infusion and testing. However, in the experiments by Swerdlow et al. (1989) and Jones et al. (1998), a 1.0 μg dose of CRF (i.c.v.) increased startle amplitude in rats that were tested 5 or 30 min after the infusion respectively. Nevertheless, time post-infusion may affect CRF-induced startle potentiation, and rodent strain may be an additional factor. Risbrough et al. (2003) infused CRF (i.c.v.) into two mouse strains 1 h prior to the start of testing the acoustic startle response. In one mouse strain, CRF increased startle amplitude at both a 1 h and a 2 h time-point post infusion, while in another strain, the effect was only seen 2 h post CRF infusion, supporting the possibility that in the present experiments, testing took place prior to the time that CRF might increase startle amplitude in the rat strains used here. The fact that CRF decreases prepulse inhibition without an increasing startle amplitude, suggests that the effect of the peptide on prepulse inhibition is not dependent on an alteration of the startle response.

As we have previously reported (Palmer et al., 2000; Conti et al., 2002), the BN strain showed diminished prepulse inhibition compared to the WKY strain. BN rats also show diminished latent inhibition (Conti et al., 2001), suggesting they are a potential model for the types of information processing deficits displayed by patients with schizophrenia. The reason for the low levels of prepulse inhibition in this strain are not known. In order to examine whether endogenous CRF has a role, rats were infused (i.c.v.) with one of two CRF receptor antagonists. Neither antagonist had an effect on prepulse inhibition in the WKY rats, and the CRF receptor antagonist, astressin failed to enhance prepulse inhibition in the BN rats. The higher dose of the CRF receptor antagonist, D-Phe, resulted in only a small, non-significant enhancement of prepulse inhibition in the BN rats, suggesting that endogenous CRF does not contribute to the low levels of prepulse inhibition seen in this strain. Another possibility is that BN rats show low prepulse inhibition because of altered monoamine neurotransmission. If so, the prepulse inhibition deficits might be attenuated by administration of monoamine receptor antagonists, including antipsychotic drugs. Feifel et al. (2004) have shown that Brattleboro rats display less prepulse inhibition than Long Evans rats, and that prepulse inhibition is increased by atypical antipsychotics, but not by haloperidol in the Brattleboro rats. The effects of both types of antipsychotic drugs in the BN rats are currently being investigated.

The CRF receptor antagonist, D-Phe, also had a significant effect on startle amplitude with the 10.0 μg dose resulting in considerably less startle in the WKY rats than saline, and both doses slightly decreasing startle amplitude in the BN strain. Astressin had no effect on startle amplitude in either rat strain. While some have shown that CRF receptor antagonists attenuate a CRF-induced increase in startle amplitude without affecting baseline startle (Swerdlow et al., 1989; Risbrough et al., 2003), others have found that a CRF receptor antagonist lowers baseline startle amplitude (Liang et al., 1992). With respect to D-Phe in particular, Schulz et al. (1995) report no effect on baseline startle amplitude in Sprague Dawley rats, yet, early in the testing session startle amplitude was lower in rats treated with D-Phe than with vehicle. D-Phe also attenuates CRF-induced locomotion (Menzaghi et al., 1994), while astressin does not (Spina et al., 2000). Nevertheless, astressin has a higher affinity than D-Phe for the CRF_1 receptor, and the two have equivalent affinity for the CRF_2 receptor (Gulyas et al., 1995; Perrin et al., 1999). In the only study to date in which the respective roles of the two CRF receptors in startle have been examined, selective antagonists for both receptors attenuated the CRF-induced increase in startle amplitude (Risbrough et al., 2003). Thus, the reason for the differential effects of astressin and D-Phe on startle amplitude found in this study requires further investigation.

CRF has been shown to increase grooming and general activity in a familiar environment (Dunn et al., 1987; Koob and Bloom, 1985; Jones et al., 1998). We have shown that high-dose CRF increases grooming in the BN, but not in the WKY strain (Conti et al., 2002). In the present study, we examined CRF-induced behavioral activation using all active behaviors, including grooming, to assess whether WKY rats fail to show behavioral activation, or simply fail to show increased grooming following CRF. Here, WKY rats spent more time engaged in active behaviors following each dose of CRF (0.1–1.0 μg) than following saline, but the effect was not dose-dependent, and maximal activation was seen at the 0.1 μg dose. In BN rats, the 0.1 μg dose of CRF had no effect above the relatively high baseline activity, while the 0.3 μg and 1.0 μg doses of CRF had significant and equivalent effects resulting in more activity than produced by these doses in the WKY rats. Thus, CRF causes behavioral activation in BN rats at doses that decrease prepulse inhibition, and behavioral activation in WKY rats at doses that do not affect prepulse inhibition, suggesting that general behavioral sensitivity cannot account for strain-dependent differences in the effects of CRF on prepulse inhibition.

In conclusion, the present findings are in accordance with our previous results (Palmer et al., 2000; Conti et al., 2002) showing that BN rats display less prepulse inhibition than WKY rats. The results also suggest that the effects of CRF on prepulse inhibition are independent of pituitary–adrenal axis activation. BN rats appear to be more sensitive to the effects of low-dose CRF on prepulse inhibition. However, administration of D-Phe, a CRF receptor antagonist, had only a small effect on prepulse inhibition in the BN rats. Thus, while exogenously administered CRF can further decrease prepulse inhibition in this strain, there appears to be a minimal, if any, contribution of endogenous CRF to the low prepulse inhibition seen in BN rats.

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