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Role of dopamine D_1 and D_2 receptors in CRF-induced disruption of sensorimotor gating

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Abstract

Corticotropin-releasing factor (CRF), a neuropeptide released during stress, has been reported to modulate startle behavior, including reducing the threshold for acoustic startle responding and reducing prepulse inhibition (PPI). The central mechanisms mediating CRF system regulation of startle and PPI are still unclear. Some antipsychotic drugs attenuate CRF-induced deficits in PPI in rats and mice. Here we tested the hypothesis that indirect activation of DA_1-receptors (D_1) and DA_2-receptors (D_2) contributes to the effects of CRF on PPI. We compared the effect of central administration of h/r-CRF (0.2–0.6 nmol) on PPI in mice with either a D_1 or D_2 receptor null mutation (knockout, KO) or in mice pretreated with D_1 or D_2 receptor antagonists SCH23390 (1 mg/kg) or haloperidol (1 mg/kg). D_1 and D_2 KO mice exhibited no significant differences in their sensitivity to CRF-induced disruptions of PPI. Similarly, neither SCH23390 nor haloperidol pretreatment altered the CRF-induced disruption in PPI, although both increased PPI at baseline. CRF-induced increases in startle also remained unchanged by any of the DA receptor manipulations. These results indicate that neither D_1- nor D_2-receptor activation is necessary for CRF to exert its effects on acoustic startle and PPI in mice.
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1. Introduction

Prepulse inhibition (PPI) is a cross-species phenomenon used as an operational measure of sensorimotor gating (Braff et al., 2001; Geyer et al., 2001; Swerdlow et al., 2001). PPI is the inhibition of the acoustic startle reflex (ASR), a contraction of the skeletal and facial muscles in response to a sudden, intense auditory stimulus, when the startling stimulus is preceded 30–300 ms by a non-startling stimulus or “prepulse” (Graham, 1975). PPI is suggested to measure pre-attentional filtering mechanisms that filter or “gate” internal and external stimuli during critical periods of information processing (Braff and Geyer, 1990). Many neuropsychiatric disorders including schizophrenia, obsessive compulsive disorder (OCD) and Huntington’s disease exhibit disrupted PPI, as do panic disorder and possibly post traumatic stress disorder (PTSD) subjects (Braff et al., 2001; Grillon et al., 1998, 1996; Ludewig et al., 2002; Swerdlow et al., 2001). Over 20 years of studies support a modulatory role of dopaminergic and serotonergic signaling mechanisms in both human and rodent PPI (for review see Geyer et al., 2001). Recently, the neuropeptide corticotropin-releasing factor (CRF) has also been shown to modulate PPI in rats and mice (Conti et al., 2002; Risbrough et al., 2004).

CRF and the related urocortin ligand family (Urocortin 1–3) mediate behavioral, autonomic, and endocrine responses to stress (Bale and Vale, 2004; Heinrichs and Koob, 2004; Dautzenberg and Hauger, 2002; Hauger et al., 2006). In addition to hypothalamic sites, CRF-containing neurons are found in a number of brainstem, limbic, and cortical nuclei

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(Asan et al., 2005; Hatalski et al., 1998; Hsu et al., 1998; Merali et al., 1998; Swanson et al., 1983; for review see Reul and Holsboer, 2002). In rodents and non-human primates, CRF receptors are expressed in neural circuits that modulate startle and mediate PPI, including nucleus accumbens, amygdala, and brain stem regions such as peduncular pontine tegemental nucleus and inferior colliculus (Van Pett et al., 2000). CRF system abnormalities have been implicated in patients with certain neuropsychiatric disorders where PPI is deficient (panic disorder, PTSD, OCD, Tourette's syndrome) (Altemus et al., 1992; Baker et al., 1999; Bremner et al., 1997; Castellanos et al., 1996; Chappell et al., 1996; Grillon et al., 1998, 1996; Hoenig et al., 2005; Holsboer et al., 1987; Kellner and Yehuda, 1999; Ludewig et al., 2005, 2002; Roy-Byrne et al., 1986; Smoller et al., 2005). CRF and urocortins acts in the brain at two distinct G-protein-coupled receptors, CRF_1 and CRF_2 (Bale and Vale, 2004; Hauger et al., 2006). In mice, transgenic-induced over-expression or exogenous administration of CRF reduces PPI (Dirks et al., 2003; Risbrough et al., 2004). CRF-induced activation of the CRF_1 receptor alone or concomitant activation of both CRF_1 and CRF_2 receptors increases startle magnitude and reduces startle threshold (Risbrough et al., 2003). However, PPI was differentially regulated whereby selective CRF_1 receptor activation decreased PPI while selective CRF_2 receptor activation increased PPI (Risbrough et al., 2004). The downstream signaling processes and neurotransmission mechanisms required for CRF receptor modulation of PPI are unknown.

There is ample evidence supporting a role for dopamine (DA) receptors in PPI (Geyer et al., 2001). Systemic administration of both direct and indirect DA receptor agonists (e.g. apomorphine and amphetamine respectively) reduces PPI (Dulawa and Geyer, 1996; Mansbach et al., 1988; Swerdlow et al., 1990). Similarly, direct infusions of DA into the nucleus accumbens decrease PPI (Swerdlow et al., 1992). Mice with excess synaptic DA levels via genetic deletion of the DA transporter exhibit robust PPI deficits (Ralph et al., 2001). Most of the above manipulations are reversed by blockade of DA_1-receptors (D_1) or DA_2-receptors (D_2) in mice (Ralph-Williams et al., 2002, 2003; Ralph et al., 2001). Hence, increased synaptic release of DA can produce significant PPI impairment, presumably via D_1 and D_2 receptor activation.

Stressors are reported to stimulate synaptic DA release and activate the DA system (Abercrombie et al., 1989; Coco et al., 1992; Deutch and Roth, 1990; Dunn, 1988; Pani et al., 2000), which may be mediated by stress-induced CRF receptor signaling (Dunn, 1988; but see Dunn, 2000). Indeed several recent reports suggest that CRF receptor activation may modulate DA release. Intracerebroventricular (ICV) CRF-administration leads to increased DA catabolites and utilization (Lavicky and Dunn, 1993; Matsuzaki et al., 1989). CRF_1 and CRF_2 receptors are localized in the ventral tegmental area (VTA) where CRF receptor agonists modulate DA release (Sauvage and Steckler, 2001; Ungless et al., 2003; Wang et al., 2005). CRF_1 receptor antagonist administration reduces cocaine-induced DA release and behavioral effects of cocaine (Lodge and Grace, 2005; Lu et al., 2003) as well as regulates dopamine D_2 receptor expression in the brain (Lawrence et al., 2005). D_2 receptors are co-localized with CRF immunoreactive neurons in the amygdala (Eliava et al., 2003). Interestingly, D_2-family receptor antagonists have been reported to attenuate CRF-induced deficits in learning (Radulovic et al., 2000) and PPI (Conti et al., 2005), suggesting that some behavioral effects of CRF may require DA receptor activation.

Thus, the primary goal of this study was to test the hypothesis that downstream DA receptor activation may be necessary for CRF-induced deficits in PPI. Because D_1 and D_2 receptors appear to be necessary for direct and indirect DA agonist induced disruption of PPI in mice, we also hypothesized that D_1 and D_2 receptors would contribute to any putative DA effects downstream of CRF system activation (Geyer et al., 2001; Ralph-Williams et al., 2003, 2002). To test our hypotheses, we utilized a complementary pharmacological and genetic approach by examining the CRF effects on startle plasticity in D_1 and D_2 receptor null mutation mice (knockout mice, KO) and after pharmacological blockade of D_1 and D_2 receptors in wild-type (WT) mice.

2. Materials and methods

2.1. Subjects

Male DA receptor D_1 and D_2 WT and KO mice (constitutive gene deletion background mice; 3–6 months of age at testing) were bred and shipped from the Oregon Health and Science University. The D_2 mice (B6.129S2-*Drd2*^{tm1Low}/*J*) were originally generated at the Oregon Health and Science University (Kelly et al., 1998) and backcrossed onto the C57BL/6J background strain for 17 generations. Stocks of D_1 mice (B6.129S4-*Drd1a*^{tm1Lcd}/*J*) (Drago et al., 1994) were obtained from the mutant mouse repository at the Jackson Laboratory (Bar Harbor, ME) and were backcrossed onto the C57BL/6J background for 10–12 generations. All mice were housed one per cage after surgery in a temperature (21–22 °C) controlled room under a reverse 12 h/12 h light cycle (lights off at 8:00 a.m.). 129T2/SVEmsJ mice (2–3 months of age at testing) from Jackson Laboratories (Bar Harbor, Maine, USA) were used for DA antagonist studies (haloperidol and SCH23390). This strain was chosen because unlike C57BL/6J mice, this strain exhibits similar PPI performance and CRF sensitivity to the D_1 and D_2 receptor WT mice (Risbrough et al., 2004; Crawley et al., 1997). A reverse light/dark cycle was used to minimize interactions with the stress of disruptions in diurnal cycles associated with testing during the sleep phase. The mice were allowed a 1-week period of acclimation to the animal room before cannulation surgery. All animal testing occurred from 10:00 a.m. to 6:00 p.m. and was conducted in accordance with the "Principles of Laboratory Animal Care" NIH guidelines, as approved by the University of California, San Diego and Veterans Affairs Medical Center animal care committees.

2.2. Surgery

Mice were anesthetized using a 90 mg/kg ketamine-2 mg/kg acepromazine cocktail and prepared with a 23 gauge 7-mm-

length unilateral guide cannula in the lateral ventricle (flat skull; anteroposterior, +0.1 mm; mediolateral, ±1.1 mm; dorsoventral, −1.5 mm below dura). Cannulae were secured with one skull screw and dental cement (Den-Mat Corp., Santa Maria, CA) and closed with a removable stylet. Mice were allowed a 5 to 7 days recovery period before testing.

2.3. Apparatus

Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) consisted of nonrestrictive Plexiglas cylinders 5 cm in diameter resting on a Plexiglas platform in a ventilated chamber. High frequency speakers mounted 33 cm above the cylinders produced all acoustic stimuli, which were controlled by SR-LAB software. Piezoelectric accelerometers mounted under the cylinders transduced the movements of the animal, which were digitized and stored by an interface and computer assembly. Beginning at startling stimulus onset, 65 consecutive 1-ms readings were recorded to obtain the average amplitude of the animal's startle response. A dynamic calibration system was used to ensure comparable sensitivities across chambers. Sound levels were measured as described elsewhere (Mansbach et al., 1988) using the A weighting scale in units of dBA sound pressure level. The chamber house-light remained off throughout all testing.

2.4. Drugs

2.4.1. Peptide infusions

ICV injections of human/rat-corticotropin-releasing factor (h/r-CRF) and subsequent histologies were as previously described (Spina et al., 2000). In brief, injections were conducted in unanesthetized mice using a 30 gauge 8 mm injector (1 mm below the tip of the guide cannulae). Injection volume was 5 μl using gravity flow. Within one week after testing was completed, mice were anesthetized and 2 μl of dye was injected via the 8 mm injector. Mice were immediately killed, and the brains were removed. As the brains were removed, presence of the dye in the fourth ventricle was noted. A coronal cut was made along the guide tract to reveal lateral and third ventricles, which were also noted for presence of dye, and brains were digitally scanned with the cut side on slides. Eight of the total 77 mice used in these studies were removed from the analysis due to incorrect cannula placement.

2.4.2. Experiment 1: h/r-CRF in D_2 WT and KO mice

Mice received either 0.6 nmol (3 μg/5 μl, ICV; Bachem, Torrance, CA; $n=8–10$) of human/rat-CRF (h/r-CRF) or artificial cerebral spinal fluid (aCSF) vehicle and were tested 1 h after injection. Pilot studies in female D_2 WT mice indicated that the 0.6 nmol dose of h/r-CRF had maximal efficacy on PPI in these mice. Mice were tested in a within-subject cross over design, with half receiving h/r-CRF and half receiving aCSF on the first test, and vice versa on the second test. Tests were separated by 1 week.

2.4.3. Experiment 2: h/r-CRF in D_1 WT and KO mice

Mice received 0, 0.2, and 0.6 nmol h/r-CRF in a within-subject latin square design ($n=9$/group), receiving each dose and vehicle once. Tests were separated from each other by at least 1 week. The lower dose (0.2 nmol) was included as we had no pilot studies on which to base our dose selection.

2.4.4. Experiment 3: haloperidol versus h/r-CRF

Mice received either 0.6 nmol h/r-CRF or aCSF vehicle 1 h before testing ($n=8$-9/group) and either sterile saline vehicle or 1 mg/kg of the D_2/D_3 receptor antagonist haloperidol (injectable preparation, Novaplus, Bedford, OH). Haloperidol was delivered by intraperitoneal (IP) injection in a 10 ml/kg volume 30 min before testing. This dose and time point were chosen because they were the most effective in attenuating PPI deficits in CRF-overexpressing (CRFOE) mice (Dirks et al., 2003).

2.4.5. Experiment 4: SCH23390 versus h/r-CRF

h/r-CRF at 0.6 nmol or aCSF vehicle was administered 1 h before startle testing ($n=7–9$/group). Sterile water vehicle or 1 mg/kg of the selective D_1 receptor antagonist SCH23390 (Tocris, Ellisville, MO) was administered by subcutaneous (SC) injection in a 10 ml/kg volume 10 min before testing. This dose and time point were chosen as they were the most effective in blocking apomorphine-induced deficits in PPI in mice (Ralph-Williams et al., 2003).

2.5. Behavioral testing

All experiments used the same acoustic startle session. The intertrial interval averaged 15 s (range of 7–23 s). During each inter-trial interval, the movements of the mice were also recorded once to measure responding when no stimulus was present. A 65 dB background was presented continuously throughout the session. After placement into the startle chambers, a 5 min acclimation period preceded testing. Startle pulses were 40 ms in duration, prepulses were 20 ms in duration, and prepulses preceded the pulse by 100 ms (onset–onset). The acoustic startle session included two blocks of different trial types. The first block tested acoustic startle response only and included 9 each of three different acoustic stimulus intensities: 90, 105, and 120 dB. The second block consisted of six startle pulse intensities (each of 105 or 120 dB) and five prepulse+pulse trials (73 and 81 dB preceding either a 105 or 120 dB pulse). In this second block, the inter-stimulus interval between prepulse and pulse onset was 100 ms.

Mice used in Experiments 3 and 4 were tested 5–7 days after surgery for baseline startle and PPI performance using the session described above. These data were used to counterbalance startle and PPI performance across drug groups. An initial test using 0.2 nmol h/r-CRF vs. haloperidol was conducted; however this dose of CRF did not produce a significant disruption of PPI (data not shown). Mice were reassigned to drug groups (equal distribution of previous treatment across groups) for Experiment 3 and again for Experiment 4. There was a 1 week washout period between drug tests.

2.6. Data analysis

The average startle magnitude over the record window (65 ms) was used for all data analysis. Percentage of PPI was

calculated using the following formula: 100 − ((average startle of the prepulse + pulse trial) / average startle in the pulse alone trial) * 100.

2.6.1. PPI analyses

In initial analyses, h/r-CRF effects on PPI were consistently independent of pulse intensity (105 or 120 dB) in all 4 experiments; hence all data and analyses shown are collapsed across pulse intensity. For Experiments 1 and 2, a 3 way ANOVA was used, with genotype (WT or KO) as a between subject factor and treatment (h/r-CRF) and prepulse intensity (73 and 81 dB) as within-subject factors. For Experiments 3 and 4, the ANOVA included treatment (h/r-CRF) and pretreatment (Haloperidol or SCH23390) as between subject factors and prepulse intensity as within-subject factors. To assess dose order effects on PPI, a 3 way ANOVA model with order, prepulse intensity and gene was used.

2.6.2. Startle analyses

For Experiments 1 and 2, a 3 way ANOVA was used, with genotype (WT or KO) as a between subject factor and treatment (h/r-CRF) and pulse intensity (90, 105 and 120 dB) as within-subject factors. For Experiments 3 and 4, the ANOVA included treatment (h/r-CRF) and pretreatment (Haloperidol or SCH23390) as between subject factors and pulse intensity as within-subject factors. To assess dose order effects on startle a 3 way ANOVA model with order, pulse intensity and gene was used. Post hoc analyses followed significant main or interaction effects as appropriate.

Table 1
Effect of h/r-CRF administration on acoustic startle magnitude (mean ± SEM) during D_1 or D_2 receptor blockade or in D_1 and D_2 receptor null mutant mice

			Vehicle	CRF [a] 1 μg	CRF [a] 3 μg
D_2	WT	p90	37 ± 11	–	75 ± 19
		p105	64 ± 15	–	138 ± 42
		p120	92 ± 13	–	209 ± 48
	KO	p90	23 ± 6	–	43 ± 14
		p105	43 ± 5	–	93 ± 30
		p120	63 ± 11	–	156 ± 30
D_1	WT	p90	41 ± 11	52 ± 13	72 ± 25
		p105	74 ± 11	99 ± 11	175 ± 58
		p120	91 ± 15	168 ± 51	293 ± 76
	KO	p90	47 ± 10	74 ± 26	83 ± 13
		p105	95 ± 11	221 ± 53	213 ± 39
		p120	132 ± 13	324 ± 52	331 ± 47
Haloperidol	Vehicle	p90	18 ± 3	–	133 ± 23
		p105	67 ± 12	–	273 ± 30
		p120	163 ± 25	–	407 ± 89
	Haloperidol	p90	19 ± 3	–	138 ± 29
		p105	59 ± 7	–	239 ± 48
		p120	169 ± 20	–	488 ± 120
SCH23390 [b]	Vehicle	p90	29 ± 9	–	209 ± 28
		p105	87 ± 18	–	338 ± 46
		p120	228 ± 54	–	693 ± 90
	SCH23390	p90	15 ± 2	–	110 ± 29
		p105	51 ± 8	–	242 ± 34
		p120	177 ± 30	–	484 ± 60

[a] Main effect of CRF in all experiments ($p < 0.01$).
[b] Main effect of SCH23390 ($p < 0.05$).

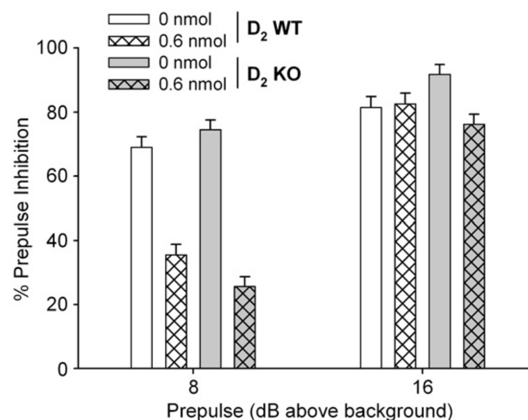

Fig. 1. Effects of h/r-CRF on PPI in D_2 wild-type (WT) and knockout (KO) mice. D_2 WT and KO mice were administered artificial cerebral spinal fluid (aCSF) and 0.6 nmol h/r-CRF (ICV, 5 μl) over 2 test sessions (1 week apart). Mice were tested for startle and PPI 1 h after CRF administration. Data are presented as mean ± pooled SEM % prepulse inhibition. CRF significantly decreased PPI across genotype at 8, but not 16, dB above background prepulse trials (see Results for details).

3. Results

3.1. Experiment 1: h/r-CRF in D_2 WT and KO mice

Administration of h/r-CRF (0.6 nmol) significantly increased acoustic startle responding in all mice at all stimulus intensities regardless of genotype (Table 1) [h/r-CRF: $F(1,16) = 14.88$, $p < 0.001$; h/r-CRF × Gene: $F(1,16) = 0.43$, n.s.]. There were no main or interactive effects of order [e.g. Order × Gene × Intensity (2,28) = 2.72, n.s.]. CRF also induced significant disruption of PPI in both KO and WT mice (Fig. 1) [h/r-CRF: $F(1,16) = 28.18$, $p < 0.001$]. PPI effects were independent of gene, although there was a trend for KO mice to exhibit greater

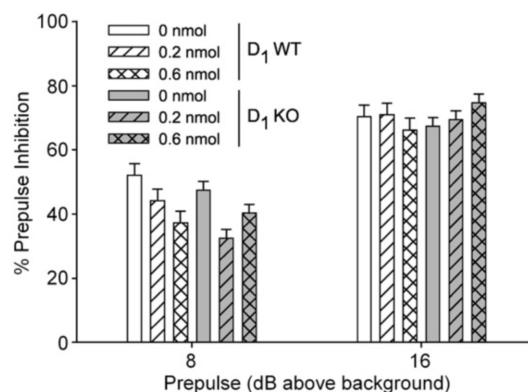

Fig. 2. Effects of h/r-CRF on PPI in D_1 wild-type (WT) and knockout (KO) mice. D_1 WT and KO mice were administered artificial cerebral spinal fluid (aCSF), 0.2 and 0.6 nmol h/r-CRF (ICV, 5 μl) over 3 test sessions (1 week apart). Mice were tested for startle and PPI 1 h after CRF administration. Data are presented as mean ± pooled SEM % prepulse inhibition. CRF significantly decreased PPI across genotype at 8 but not 16 dB above background prepulse trials (see Results for details).

CRF-induced PPI disruption compared to WT mice [h/r-CRF × gene, $F(1,16)=3.08$, $p=0.09$]. On closer inspection of the data, the main effect of CRF was largely due to a significant disruption of PPI at the 8 dB but not 16 dB above background trials (73 and 81 dB respectively) (Fig. 1) [h/r-CRF × prepulse, $F(1,16)=28.32$, $p<0.001$]. There were no main or interactive effects of order [e.g. Order × Gene × Prepulse $F(1,14)<1$, n.s.].

3.2. Experiment 2: h/r-CRF in D_1 WT and KO mice

Overall CRF treatment increased startle [h/r-CRF: $F(2,32)=10.39$, $p<0.001$] independently of the presence of the D_1-receptor [Table 1; h/r-CRF × gene: $F(2,32)=1.43$, n.s.]. Again, PPI was significantly reduced in response to h/r-CRF treatment at the 8 dB above background prepulse trials (Fig. 2) [h/r-CRF × repulse: $F(2,32)=3.41$, $p<0.05$]. CRF-induced disruption of PPI was not found to depend on D_1 genotype [h/r-CRF × gene: $F(2,32)<1$, n.s.]. Similarly to Experiment 1, there were no main or interactive effects of order for either PPI analyses [e.g. Order × Gene × Intensity $(2,12)<1$, n.s.] or startle analyses [e.g. Order × Gene × Intensity $(4, 24)<1$, n.s.].

3.3. Experiment 3: haloperidol versus h/r-CRF

The data from Experiment 1 led to the hypothesis that the D_2 receptor is not essential for the effects of CRF. We investigated whether we could replicate these data with a pharmacological D_2 receptor blockade. Haloperidol, an antagonist at D_2 and D_3 receptors, was chosen as it had previously been reported to attenuate the PPI deficits in CRFOE mice (Dirks et al., 2003). The 0.6 nmol dose of h/r-CRF increased startle magnitude at all intensities [h/r-CRF: $F(1,29)=19.11$, $p<0.001$]. CRF treatment groups exhibited decreased PPI at the 8 dB above background prepulse trials (Table 2) [h/r-CRF × prepulse, $F(1,29)=7.33$, $p<0.05$]. As seen in D_2 KO mice, D_2 blockade via haloperidol had no effect on CRF-induced decreases in PPI (Table 2) [h/r-CRF × haloperidol: $F(1,29)<1$, n.s.], although haloperidol did significantly increase PPI independent of CRF at some trial types [Haloperidol × prepulse × pulse intensity: $F(1,29)=6.82$, $p<0.05$], confirming previous reports (Ouagazzal et al., 2001).

Table 2
Effect of pharmacological blockade of D_1 and D_2 receptors on CRF-induced deficits in %PPI (data shown as mean ± SEM for 8 dB and 16 dB above background prepulse trials)

Prepulse intensity	8 dB		16 dB	
	Vehicle	CRF [a]	Vehicle	CRF
Vehicle	47±7	39±9	62±6	64±7
Haloperidol	58±6	38±7	67±4	64±4
Vehicle	47±9	33±6	62±7	59±6
SCH23390 [b]	63±6	49±6	76±3	73±3

[a] Main effect of CRF on PPI at 8 dB above background trials in both Haloperidol and SCH23390 studies.
[b] Main effect of SCH23390 treatment (see text for details).

3.4. Experiment 4, SCH23390 versus h/r-CRF

CRF administration increased startle significantly at all startle intensities (Table 1) [h/r-CRF, $F(1,29)=63.35$, $p<0.001$], independently of SCH23390 treatment [h/r-CRF × SCH23390, $F(1,29)=2.63$, n.s.]. SCH23390 treatment decreased startle independently of the presence of CRF [SCH23390, $F(1,29)=7.30$, $p<0.05$]. CRF disrupted PPI at the 8 dB above background dB prepulse trials (Table 2) [h/r-CRF × prepulse, $F(1,29)=4.64$, $p<0.05$]. SCH23390 administration did not reverse the CRF-induced PPI deficits [h/r-CRF × SCH23390, $F(1,29)<1$, n.s.] although it did increase overall PPI-performance in both aCSF and CRF treatment groups [SCH23390, $F(1,29)=10.49$, $p<0.01$].

4. Discussion

The objective of the present study was to investigate the role of D_1 and D_2 receptors in CRF-induced deficits in PPI. The present studies used a complementary approach to address this question, by determining the effects of CRF in mice with either genetic null mutation of the D_1 or D_2 receptor, and in mice treated with selective D_1-family and D_2-family receptor antagonists. We found that acute CRF administration increased startle reactivity and reduced PPI when either the D_1 or the D_2 receptor was genetically deleted or pharmacologically blocked. These results indicate that neither D_1 nor D_2-receptor activation is necessary for CRF to exert its effects on acoustic startle and PPI.

Several studies have shown that central CRF administration and stress can influence synaptic DA release in rodents (see Introduction). In addition, D_2 receptors appear to contribute to the PPI disruption induced by DA release in mice. For example, D_2 KO mice or mice treated with the D_2-family antagonist raclopride are insensitive to amphetamine-induced PPI disruption, while raclopride administration reverses the PPI deficits observed in DA transporter KO mice (Ralph-Williams et al., 2003, 2002). Recent data indicate that haloperidol attenuates the PPI deficits observed in CRFOE mice with constitutive CRF-overexpression (Dirks et al., 2003). Haloperidol treatment can also reverse CRF-induced disruption in PPI in Wistar Kyoto but not Brown Norway rats (Conti et al., 2005). Hence it was surprising that neither haloperidol nor D_2 receptor gene deletion attenuated the effects of acute CRF on PPI in mice. The haloperidol dose used here is the same dose shown to be effective in reducing the magnitude of PPI disruption observed in CRFOE mice and CRF-treated rats (Conti et al., 2005; Dirks et al., 2003). The discrepancy between our findings and previous studies showing that haloperidol attenuates CRF-dependent PPI disruption may be explained by the following: (1) haloperidol is only effective in cases were CRF is chronically released or (2) haloperidol's effect is dependent upon strain and/or species. Although CRFOE mice have been shown to exhibit alterations in the CRF system, it is unknown if these mice have abnormal functioning of their DA systems (Kozicz et al., 2004; Peeters et al., 2004; Weninger et al., 2000). A very recent study has indicated that D_2 receptors are upregulated

after chronic CRF_1 receptor antagonism (Lawrence et al., 2005), suggesting that CRF receptor activity can modulate D_2 receptor signaling. Haloperidol has also been reported to attenuate acute CRF effects on PPI in rats (Conti et al., 2005), although this effect was strain dependent. Furthermore, other evidence suggests that D_2 receptor agonist and antagonist effects on PPI depend on strain in mice (McCaughran et al., 1997; Ralph and Caine, 2005). In the present studies we used 129T2/SVEmsJ mice, whereas CRFOE mice were on a C57BL/6J background. Thus, haloperidol effects on CRF-induced deficits in PPI may be dependent upon mouse strain. It should also be noted that in the present studies we did not see a deficit in PPI in the D2 KO mice, which has been observed in some (Ralph-Williams et al., 2002) but not all (Ralph et al., 1999) studies. It is not clear if this inconsistent phenotype is due to continuous backcrossing to C57BL/6J background over the years, however our experience is that these mice generally do not show a deficit across cohorts (unpublished observations).

Although the dose of haloperidol chosen in the present studies has been shown to be efficacious in CRFOE mice and CRF-treated rats, it is possible that higher doses of haloperidol would have been efficacious. In the present studies, we observed a slight but significant increase in PPI in the haloperidol treatment groups. Hence, increasing the dose would likely increase PPI regardless of CRF treatment, as well as reduce startle responding (Dirks et al., 2003; Ouagazzal et al., 2001), reducing the interpretability of such a study. In agreement with the present haloperidol data, we found that D_2 KO mice exhibit normal or even slightly potentiated effects of CRF on PPI. These data would indicate that D_2 receptor signaling is not necessary for CRF effects on PPI. Although haloperidol attenuated CRFOE PPI deficits, clozapine and risperidone, also D_2-family antagonists (among other activity), did not (Dirks et al., 2003). Taking these data together, we suggest that haloperidol effects on CRF-induced disruptions in PPI may be via activity at other receptors.

In mice, activation of D_1 receptors could also contribute to putative DA-mediated disruptions of PPI (Ralph-Williams et al., 2003, 2002; Ralph and Caine, 2005). In the present studies, CRF significantly reduced PPI in D_1 KO mice and in the presence of behaviorally active doses of the D_1 antagonist SCH23390. These data support the conclusion that D_1 receptors are not required for CRF effects on startle or PPI. D_1 KO mice did appear to be less sensitive to the effects of the high dose (0.6 nmol) of h/r-CRF (Fig. 2). In contrast, pharmacological blockade of D_1 with SCH23390 had no specific effects on this dose of CRF in WT mice. This 1 mg/kg dose of SCH23390 significantly increased PPI and reduced startle in WT mice, which we have not observed in other strains (Ralph et al., 2001). We chose this dose as it has been shown to attenuate the locomotor phenotype in DAT KO mice as well as reverse apomorphine- and cocaine-induced decreases in PPI (Ralph-Williams et al., 2003, Geyer unpublished observations; Ralph et al., 2001). While our study shows that blockade of either D_1 or D_2 receptors does not reverse CRF-induced PPI deficits, it could be argued that either D_1 or D_2 receptors may still be sufficient for CRF-induced deficits in PPI, hence blocking only one receptor at a time will have no effect. In such a case, simultaneous blockade of both receptor subtypes would reverse CRF-induced reductions in PPI. We have found however, that when given in combination, haloperidol and SCH23390 do not reverse CRF effects on PPI (Risbrough, Caldwell, Geyer unpublished observations). Thus, taken together, it seems that CRF effects on PPI and startle do not require activation of D_1 or D_2 receptors.

Previously, we and others have shown that h/r-CRF decreases PPI independent of prepulse and pulse intensity (Risbrough et al., 2004). In our experiments in mice and in studies with certain rat strains, however, h/r-CRF had consistent effects on PPI only at the lower prepulse-intensity trials (Conti et al., 2005, 2002). The lack of effect of h/r-CRF at the higher prepulse intensities in these studies may be due to differences in baseline performance. In the present studies, we found an average of 70% inhibition across all trials, whereas using the same testing session Risbrough et al. (2004) showed a 40–50% average across the trial types. The high intensity prepulse trials (81 dB) in the present studies may thus be less vulnerable (inhibition was up to 80% at these trials) to disruption than the lower intensity and presumably less salient 73 dB prepulse trials (Figs. 1 and 2). Thus lower prepulse intensities may produce a “threshold” of inhibition that is more easily disrupted by CRF administration. The dose response of h/r-CRF effects on PPI was also slightly shifted compared to previous reports in mice and in rats (Conti et al., 2002; Risbrough et al., 2004), with the present studies requiring higher doses of h/r-CRF to induce significant disruption of PPI (Risbrough et al., 2004). It is unclear if this pattern is due to potential batch differences in the peptide, or in the strains used (C57BL/6J from Jackson and 129SvEv from Taconic vs. a congenic C57BL/6J bred at OHSU and 129T2/SVEmsJ mice in the present studies). Despite these small discrepancies in parameters and doses, however, the CRF-induced reduction in PPI and increase in startle appears to be reasonably replicable across mouse strains.

The administration of CRF robustly increased startle in all of our experiments. Administration of haloperidol or SCH23390 as well as DA receptor gene manipulation did not affect CRF-induced increases in startle (Table 1). These results confirm earlier experiments in which CRF induces large increases in startle magnitude in both rats and mice (Liang et al., 1992; Risbrough et al., 2003, 2004; Swerdlow et al., 1989, 1986). In rats, CRF effects on startle have been localized to a hippocampal–septal-bed nucleus stria terminalis circuit (for review see Davis et al., 1997), which expresses both CRF_1 and CRF_2 receptors (Van Pett et al., 2000). CRF-induced increases in startle are blocked by steroids such as progesterone treatment and alphaxalone (Swerdlow and Britton, 1994; Toufexis et al., 2004), blocked by GABAergic activation via chlordiazepoxide administration (Swerdlow et al., 1989), and enhanced by corticosterone and vasopressin treatment (Lee et al., 1994; Pelton et al., 1997). Recently, Meloni et al. (2006) reported that SCH23390 attenuated CRF-induced increases in startle in rats (PPI was not tested). It is unclear if the discrepancy between our findings (no effect of SCH23390 or genetic deletion of D_1) and those of Meloni et al. (2006) is due to species (rat vs. mice) or

methods of administration of SCH23390. In rats, SCH23390 induced a U-shaped dose response curve, with the high dose of SCH23390 (0.5 mg/kg) having no effect, while lower doses reduced CRF effects on startle (Meloni et al., 2006). It is thus possible that lower doses of SCH23390 would have been more effective in the present studies. A difference in the timepoint of administration before CRF administration may also account for the discrepancies seen across these studies. Nevertheless, in light of our present finding in mice that full blockade of D_1 via null mutation has no effect on CRF-induced alterations in startle behaviors, a positive result of SCH23390 might have called into question the selectivity of SCH23390 for D_1 rather than indicate that D_1 receptors are required for CRF effects on startle in mice. SCH23390 has been shown to be active at other receptors (e.g. potent efficacy at 5-HT_{2C} (Millan et al., 2001) and possibly D_5 receptors (Centonze et al., 2003)). Indeed, the 0.05 and 0.1 mg/kg doses of SCH23390 that were effective in blocking CRF-induced increases in startle in rats (Meloni et al., 2006) have been shown to have behavioral effects in D_1 KO mice, indicating that at least in mice, SCH23390 has functional activity at receptors other than D_1 (Centonze et al., 2003). A more general explanation of why D_1 and D_2 blockade had no effect on CRF-induced effects on startle in the present studies, but has been shown to be effective in rats (Meloni et al., 2006; Conti et al., 2005) may be due to species differences in dopamine system control of startle behaviors. We have previously shown that D_1 and D_2 agonist effects on PPI in mice do not match the pattern of effects seen in rats (Ralph-Williams et al., 2003, 2002; Wan et al., 1996). Hence, it is possible that our discrepant findings are due to differences in DA control of startle behaviors across species.

Besides the enervation of dopaminergic systems, CRF also interacts with other monoamine systems that modulate sensorimotor gating (Geyer et al., 2001; Sauvage and Steckler, 2001). CRF fibers innervate brain structures such as the serotonergic dorsal raphe nucleus (DRN) (Kirby et al., 2000; Lowry et al., 2000; Pernar et al., 2004) and the noradrenergic locus coeruleus (LC-NE) (Dunn et al., 2004; Emoto et al., 1993; Lavicky and Dunn, 1993; Matsuzaki et al., 1989; Pernar et al., 2004; Valentino et al., 2001). ICV administration of CRF increases NE turnover and utilization in the medial frontal cortex and hippocampus (Lavicky and Dunn, 1993; Matsuzaki et al., 1989; Zhang et al., 1998), and β-adrenergic receptors have been shown to be involved with stress- and CRF-induced effects on defensive behavior (Gorman and Dunn, 1993; Yang and Dunn, 1990). CRF modulation of glutamate neurotransmission, perhaps at the amygdala, may also be a potential mechanism for CRF effects on startle behavior (Liu et al., 2004, 2005; Swerdlow et al., 2001). Additional research is required to explore these alternative mechanisms for CRF-induced alterations in startle behavior.

In summary, we found that neither gene null mutation nor pharmacological blockade of DA D_1 or D_2 receptors significantly affected CRF-induced decreases in PPI and increases in startle in mice. The present studies indicate that CRF-induced disruptions of PPI do not require D_1 or D_2 receptor activation in mice.

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