A selective, non-peptide CRF receptor 1 antagonist prevents sodium lactate-induced acute panic-like responses

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Abstract
Corticotropin releasing factor (CRF) is implicated in a variety of stress-related disorders such as depression and anxiety, and blocking CRF receptors is a putative strategy for treating such disorders. Using a well-studied animal model of panic, we tested the efficacy of JNJ19567470/CRA5626, a selective, non-peptidergic CRF type 1 receptor (CRF1) antagonist (3, 10 and 40 mg/kg intraperitoneal injection), in preventing the sodium lactate (NaLac)-induced panic-like behavioural and cardiovascular responses. Adult male rats with chronic reduction of GABA levels (by inhibition of GABA synthesis with l-allyglycine, a glutamic acid decarboxylase inhibitor) in the dorsomedial/perifornical hypothalamus are highly anxious and exhibit physiological and behavioural responses to intravenous NaLac infusions similar to patients with panic disorder. These ‘panic-prone’ rats pre-treated with vehicle injections displayed NaLac-induced increases in autonomic responses (i.e. tachycardia and hypertensive responses), anxiety-like behaviour in the social interaction test, and flight-like increases in locomotor activity. However, systemically injecting such panic-prone rats with the highest dose of CRF1 receptor antagonist prior to NaLac infusions blocked all NaLac-induced behaviour and cardiovascular responses. These data suggest that selective CRF1 receptor antagonists could be a novel target for developing anti-panic drugs that are as effective as benzodiazepines in acute treatment of a panic attack without the deleterious side-effects (e.g. sedation and cognitive impairment) associated with benzodiazepines.

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Introduction
Panic disorder is a severe anxiety disorder that is characterized by recurrent panic attacks (APA, 1994). One of the most consistent abnormalities in panic disorder is a vulnerability to displaying panic attacks following exposure to mild interoceptive cues such as hypertonic 0.5 M sodium lactate (NaLac) infusions (Liebowitz et al. 1984, 1985), CO2 inhalations (Gorman et al. 1994), and doxapram (Gutman et al. 2005). Therefore, the initial pathology in these patients appears to be an alteration somewhere in the central neural pathways regulating normal panic responses, thus rendering the patients susceptible to unprovoked panic symptoms when exposed to ordinarily mild interoceptive stressors (Vickers & McNally, 2005). Serotonin reuptake inhibitors are the first-line drugs for panic disorder, but it often takes several weeks for full response with significant drop-outs due to side-effects (for review see Cloos & Ferreira, 2009). Benzodiazepines are effective with rapid effects, but side-effects such as sedation and dependence are common (Baldwin et al. 2005; Bandelow et al. 2008; Cloos & Ferreira, 2009; Nutt et al. 2002). Therefore, there is a great need for rapidly effective anxiolytic agents without the typical benzodiazepine side-effects.

The neuropeptide corticotrophin-releasing factor (CRF) discovered in 1981 by Vale and colleagues (1981), in addition to its endocrine functions, is synthesized and released in other extra-hypothalamic
brain regions (Keegan et al. 1994; Swanson & Simmons, 1989), where it acts as a neurotransmitter/neuromodulator to coordinate behavioural and autonomic responses to stress. So far, two CRF receptor subtypes, CRF₁ and CRF₂, have been identified (Chalmers et al. 1995; Dautzenberg & Hauger, 2002; De Souza et al. 1985; Perrin & Vale, 1999). Increasing evidence suggests that CRF may play a critical role in mediating some types of anxiety responses, and that the CRF system may be a potential therapeutic target for the treatment of anxiety disorders (Baldwin et al. 1991; Britton et al. 1982, 1985; Cole & Koob, 1988; Dunn & Bertridge, 1987; Dunn & File, 1987; Liang & Lee, 1988; Liang et al. 1992; Swerdlow et al. 1986; Takahashi et al. 1989). Central injections of CRF mobilize ‘panic/defence’ responses (Brown et al. 1988; Ku et al. 1998) and injections of the panic-inducing agent doxapram activate CRF neurons (Choi et al. 2005). Consistent with these preclinical findings, clinical evidence suggests that polymorphisms in CRF₁ receptor gene may be associated with panic (Keck et al. 2008) and the CRF system may be an important therapeutic target for panic disorder (Risbrough & Stein, 2006).

Although several highly specific, non-peptide, small molecule, CRF₁ receptor antagonists with good solubility, bioavailability and brain penetration have shown anxiolytic-like effects (Gehlert et al. 2005; Keck et al. 2001; Keller et al. 2002; Steckler et al. 2006), none have been tested for their anti-panic effects. Therefore, we sought to determine the anti-panic efficacy of a selective CRF₁ receptor antagonist (Steckler et al. 2006) utilizing a well characterized animal model of NaLac-induced panic-like response (Johnson & Shekhar, 2006; Johnson et al. 2008b; Shekhar & Keim, 1997, 2000; Shekhar et al. 1996, 2006).

Methods and materials

Animals and housing conditions

All experiments were conducted on adult male Sprague–Dawley rats (300–350 g), which were purchased from Harlan Laboratories (USA) and were housed individually in plastic cages under standard environmental conditions (22 °C, 12-h light/dark cycle, lights on 07:00 hours) for 7–10 d prior to surgical manipulation. Food and water were available ad libitum. Animal care procedures were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication no. 80–23) revised 1996 and the guidelines of the IUPUI Institutional Animal Care and Use Committee.

Surgical procedures and osmotic minipump infusions

Prior to and during surgery, rats were anaesthetized with a nose cone connected to an isoflurane system (MGX Research Machine, USA). Rats were fitted with femoral arterial catheters for measurement of mean arterial blood pressure (MAP) and heart rate (HR) and with venous catheters for intravenous (i.v.) infusions, as previously described (Shekhar et al. 1996).

Cardiovascular responses (i.e. MAP and HR) were measured by a femoral arterial line connected to a telemetric probe which contained a pressure transducer [cat. no. C50-PXT, Data Science International (DSI), USA]. DSI DATAQUEST software was used to monitor and record MAP and HR. MAP and HR were recorded continuously in freely moving conscious rats and are expressed as a 20-min time-course. The data reported are changes in HR and MAP from the average of the baseline (t−5 to t−1) from each rat.

After 3 d of recovery, animals were tested for baseline cardiovascular responses to lactate (see below). Following baseline testing, rats were anaesthetized as stated previously and 26-gauge T-shaped cannulae (cat. no. 3260PG, Plastics One Inc., USA) were directed at cardio-excitatory regions of the dorsomedial/perifornical hypothalamus (DMH/PeF; Samuels et al. 2004) based on the following coordinates (from bregma: 1.2 mm posterior, +2.1 mm lateral, +9.1 mm ventral and adjusted for approaching at a 10° angle towards the midline with the stereotaxic incisor bar elevated 5 mm above the inter-aural line) and cemented into place as described previously (Shekhar et al. 1996). The 22-gauge side arm was then attached, via PE-60 tubing, to an osmotic minipump [prefilled with L-allyglycine (L-AG) solution] and sutured into place subcutaneously at the nape of the neck (DURECT Corporation, model no. 2002). The concentration of the solutions was such that 3.5 nmol/0.5 μl per hour of L-AG or D-AG was infused continuously into the DMH region for the remainder of the given experiment.

Previous studies have determined that the dose of L-AG utilized here reduces local GABA concentrations by approximately 60% following unilateral infusions (Abshire et al. 1988; Shekhar & DiMicco, 1987; Shekhar & Keim, 1997; Shekhar et al. 1996, 2006) and supported by immunohistochemistry (Johnson & Shekhar, 2006) increases anxiety-like behaviour [i.e. as measured by the social interaction (SI) test and elevated plus-maze (EPM)] without increasing cardio-respiratory responses (Johnson & Shekhar, 2006; Johnson et al. 2008b; Shekhar & Keim, 1997; Shekhar et al. 1996, 2006).
Intraperitoneal (i.p.) injections of JNJ19567470/CRA5626, a selective CRF<sub>1</sub> receptor antagonist

Five days following l-AG infusion onset, in a counterbalanced design, rats received an i.p. injection of either CRF<sub>1</sub> receptor antagonist, JNJ19567470/CRA5626 [3 mg/kg (n = 4), 10 mg/kg (n = 11), 40 mg/kg (n = 7)], or vehicle (0.2 ml/100 g volume DMSO, n = 15)] 30 min prior to NaLac challenge. Steckler and colleagues have previously shown that the JNJ19567470/CRA5626 compound used here exhibits the following: (1) affinity and high selectivity (some weak affinity is noted for the δ opiate receptors) for recombinant and native CRF<sub>1</sub> receptors expressed in brain tissue and cells that does not appear to be species specific; (2) acts as a CRF<sub>1</sub> receptor antagonist in AtT20 cells in vitro; (3) dose-dependently occupies CRF<sub>1</sub> receptors in the brain ex vivo following peripheral administration; and (4) is highly concentrated in brain and plasma levels after oral administration (Steckler et al. 2006).

I.p. injections of alprazolam, a benzodiazepine

Five days following l-AG infusion onset, in a counterbalanced design, rats received an i.p. injection of either alprazolam [3 mg/kg (n = 6), cat. no. 1960, Tocris (UK), in 0.2 ml/100 g volume DMSO], or vehicle (0.2 ml/100 g volume DMSO, n = 6) 30 min prior to NaLac challenge.

Description of NaLac infusion

Five days following stereotaxic osmotic minipump implantation, cardiovascular responses were recorded continuously until a 5-min stable baseline was achieved. Rats were then given their assigned i.v. infusion [NaLac or saline (when applicable)] and cardiovascular and activity data were recorded for 15 min following onset of infusion, as described previously (Shekhar et al. 1996). Thus, freely moving rats in home cages were given i.v. infusions of 0.5 ml NaLac in vehicle (10 ml/kg over 15 min), similar to clinical lactate infusions (Liebowitz et al. 1986).

SI test

The SI test of experimental anxiety-like behaviour in rats (File, 1980), modified for use in our laboratory has been described previously (Sanders & Shekhar, 1995; Shekhar & Katner, 1995). The apparatus itself consists of a solid wooden box with an open roof approximately 0.9 m long x 0.9 m wide with walls 0.3 m high. All behavioural tests were videotaped with a camera above the box. The ‘experimental’ rat and an unfamiliar ‘partner’ rat were both allowed to individually habituate to the box for a 5-min period 24 h prior to each SI test. During the SI test, the two rats were placed together in the centre of the box, and the total duration (s) of non-aggressive physical contact (grooming, sniffing, crawling over and under, etc.) initiated by the ‘experimental’ rat is quantified over a 5-min period. A baseline SI test was performed ≥72 h after i.v. catheterization, but prior to osmotic minipump implantation. Another SI test was performed 5 d following minipump infusions and immediately following saline or NaLac infusions. Videotaped sessions were scored at a later time (by S.D.F.), who was blind to any drug treatment.

Histology

Following experiments, all rats were anaesthetized and decapitated and their brains removed and frozen; the brains were sectioned (30 μm) and stained with Neutral Red for determination of injection cannulae placements.

Statistical analyses

Each dependent variable for in-vivo analyses (i.e. SI duration, activity, HR, MAP) was analysed using one-way ANOVA with repeated measures with drug treatment as main factor and time as the repeated measure. Levene’s Test of Equality of Error Variance was also performed in order to determine equal variances in the groups. When there were equal variances in the groups and in the presence of significant main effects, between-subject post-hoc tests were conducted using a parametric Tukey’s test and within-subject time effects were assessed using Dunnett’s one-way analysis with the minute prior to i.v. infusion used as control. When there were unequal variances in the groups, post-hoc tests were conducted using a non-parametric Kruskal–Wallis test. Statistical significance was accepted as p < 0.05. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., USA) and Systat 5.02 for Windows (Systat Inc., USA), and all graphs were generated using SigmaPlot 2001 for Windows (SPSS Inc.), figure-plate illustrations were done using CorelDraw version 12 for Windows.

Results

Histological verification of hypothalamic infusion sites

All minipump cannulae placements resided in regions of the DMH/PeF known to be cardio-excitory.
(Samuels et al. 2004; Shekhar, 1993; Shekhar & DiMicco, 1987) (see Fig. 1). No significant tissue damage at the site of implantation was noted due to the 26-gauge cannula (0.46 mm o.d.). Previous immunohistochemical analyses of the total numbers of Neu-N-positive neurons as well as N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor immunoreactive neurons in the DMH/PeF from rats that had similar L-AG infusions showed no difference between the side of the DMH which had the cannula track compared to the intact DMH/PeF on the contralateral side (Johnson & Shekhar, 2006).

**Effects of prior systemic injections of CRF₁ antagonist on behavioural and cardiovascular responses in L-AG-treated rats following NaLac challenge**

Intraperitoneal injections of the highest dose of JNJ19567470/CRA5626 CRF₁ receptor antagonist 30 min prior to the lactate challenge attenuated lactate-induced ‘anxiety’, i.e. reduced social interaction time during the 5-min test ($F_{1,10} = 6.5, p = 0.000$; Fig. 2a), and changes (from baseline) in HR (treatment x time effect: $F_{57,589} = 1.4, p = 0.037$; Fig. 2b), and MAP (treatment x time effect: $F_{57,589} = 2.4, p = 0.000$; Fig. 2d) in panic-prone rats. As predicted, cardio-excitatory [MAP ($F_{19,239} = 3.9, p < 0.001$); HR ($F_{19,239} = 3.9, p < 0.001$)] and ‘flight’ responses (shown as increased locomotor activity) were noted in vehicle-treated panic-prone rats following the lactate challenge (time effect: $F_{28,315} = 1.7, p = 0.019$). However, unlike the vehicle controls, no change in MAP ($F_{19,120} = 1.1, p = 0.388$), HR ($F_{19,120} = 0.6, p = 0.867$), or activity ($F_{19,120} = 1.0, p = 0.413$) was noted over 5-min baseline +15 min of i.v. infusions in rats receiving the highest dose (40 mg/kg) of CRF₁ receptor antagonist. Although no increases in locomotor activity were noted in the 10-mg and 40-mg doses of CRF₁ receptor antagonist, a significant increase in locomotor activity was noted in the 3-mg/kg dose of CRF₁ receptor antagonist that was of longer duration than vehicle controls (see symbols in Fig. 2c denoting between-subject differences from the vehicle-treated group as revealed by Tukey’s HSD post-hoc test). There was no significant differences in baseline (5 min prior to i.v. infusion) activity ($F_{3,11} = 0.6, p = 0.595$), HR ($F_{3,11} = 1.4, p = 0.246$) or MAP ($F_{3,11} = 0.8, p = 0.508$) between groups (see legends to figures with baseline activity, HR and MAP ± S.E.M.). Nor did any dose of the CRF₁ receptor antagonist have

![Fig. 1. Schematic representation of the infusion sites of L-allylgycine (L-AG; a GABA synthesis inhibitor) as determined by histology. Infusion cannula placements for L-AG infusions are illustrated as solid circles (●) for CRF₁ receptor antagonist experiments and open circles (○) for alprazolam experiments. Illustrations of coronal brain sections are based on a standard stereotactic rat brain atlas (Paxinos & Watson, 1997). Numbers below each section indicate the distance posterior from bregma; the scale on the right of each section represents the distance ventral from bregma (in mm). Solid lines represent white-matter tracts and dashed lines illustrate subdivisions of brain. The grey shaded area represents the dorsomedial and perifornical hypothalamus (DMH/PeF) region. Abbreviations: 3 V, 3rd ventricle; f, fornix; mt, mammillothalamic tract. Scale bar, 800 μm.](http://ijnp.oxfordjournals.org/)

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significant effects on HR, MAP or activity over time prior to the onset of NaLac challenge (assessed 10 min following injection, with 10 min allowed for rats to regain baseline value following i.p. injection; Fig. 3a: HR (3 mg, \( F_{1,26} = 0.4, \ p = 0.692; \) 10 mg, \( F_{1,18} = 1.5, \ p = 0.231; \) 40 mg, \( F_{1,12} = 0.3, \ p = 0.599)\); Fig. 3b: MAP (3 mg, \( F_{1,60} = 0.2, \ p = 1.000; \) 10 mg, \( F_{1,18} = 0.9, \ p = 0.395; \) 40 mg, \( F_{1,12} = 0.8, \ p = 0.640)\); Fig. 3c: activity (3 mg, \( F_{1,60} = 0.6, \ p = 0.850; \) 10 mg, \( F_{1,18} = 0.7, \ p = 0.814; \) 40 mg, \( F_{1,12} = 0.8, \ p = 0.640)\). There were no differences in \( t = 20 \text{ min} \) to \( t = 15 \text{ min} \) baseline in HR (\( F_{1,26} = 0.2, \ p = 0.695 \)) or MAP (\( F_{1,26} = 0.7, \ p = 0.553 \)), but activity was higher at baseline in the 3-mg group, compared to vehicle group (\( F_{1,26} = 4.4, \ p = 0.011 \)). Two telemetry probes malfunctioned due to low batteries in the 1-AG/vehicle groups, which did not impact social interaction data which remained (\( n = 15 \)), but reduced the number for the activity, HR and MAP by two for the 1-AG/vehicle group (i.e. \( n = 11 \)).
Effects of a prior treatment with alprazolam on behavioural and cardiovascular responses in t-AG-treated rats following NaLac challenges

Intraperitoneal injections of alprazolam (3 mg/kg) 30 min prior to lactate challenge attenuated lactate-induced reduction in social interaction time ($F_{1,19} = 19.9, p = 0.000$; Fig. 4a) and changes (from baseline) in HR (treatment $\times$ time effect: $F_{1,19} = 2.0, p = 0.011$; and a within-subjects increase in HR post-i.v. infusion in t-AG + vehicle group (time effect: $F_{1,19} = 0.6, p = 0.70$; Fig. 4b) in panic-prone rats. A homogeneity test for variance (i.e. Levene’s Test of Equality of Error Variances) revealed unequal variance in the HR over time in the t-AG + vehicle group ($F_{1,19} = 3.3, p < 0.001$), therefore a non-parametric Kruskal–Wallis post-hoc test was used to compare pre-i.v. infusion HR ($-1 \text{ m}$) to each post-i.v. infusion HR. Panic-prone rats displayed no change in general activity (treatment $\times$ time effect: $F_{1,19} = 0.6, p = 0.737$; Fig. 4c) or MAP (treatment $\times$ time effect: $F_{1,19} = 0.4, p = 0.987$; Fig. 4d) following NaLac. There was no significant differences in baseline activity ($t_s = -0.2, p = 0.853$), HR ($t_s = -0.3, p = 0.770$) or MAP ($t_s = -0.04, p = 0.970$) in any rat prior to NaLac infusion (see figure legends with baseline activity, HR and MAP $\pm$ S.E.M.).

Discussion

In the present study, systemically injecting panic-prone rats with the highest dose of CRF$_1$ receptor antagonist (JNJ19567470/CRA5626), prior to NaLac, blocked all NaLac-induced panic-like behaviour and cardiovascular responses. Pretreating rats with the CRF$_1$ antagonist not only had acute anti-panic effects, but also did not alter baseline levels of locomotor activity and actually increased the duration of social explorations, suggesting lack of any sedating effects, a common side-effect of benzodiazepines (Baldwin et al. 2005; Bandelow et al. 2008; Nutt et al. 2002). The rats receiving the 3 mg/kg dose of CRF$_1$ receptor antagonist did show an increase in locomotor behaviour following NaLac which lasted longer than the vehicle group. However, the 3 mg/kg group had no increases in anxiety behaviour, HR or MAP responses to NaLac compared to the vehicle group. By itself, the locomotor data is not a measure of anxiety or panic (e.g. increases in exercise would not be panic or anxiety). However, an increase in locomotion in the presence of anxiety behaviour (here we used a social anxiety test) and panic-associated neuro-excitatory responses, is potentially indicative of flight-associated panic behaviour. Overall, this suggests that the 3 mg/kg dose was
not exacerbating anxiety/panic responses to the lactate challenge. The CRF$_1$ antagonist also did not have any effect on baseline blood pressure, HR or activity over time in the interval between CRF$_1$ antagonist injections and NaLac infusion, although there is a possibility that the CRF$_1$ receptor antagonist was decreasing cardiovascular and general activity measures selectively at the time of NaLac infusion with a net effect being blockade of the panic-like responses during NaLac infusion. However, previous studies have shown that the effects of this CRF$_1$ antagonist is dependent on the behavioural state of the rodents (for review see Hokfelt et al. 2003). Overall, these data suggest that blocking CRF$_1$ receptors may provide an acute anti-panic drug similar to high potency benzodiazepines such as alprazolam, without the accompanying adverse effects.

Although there are significant limitations in being able to predict the efficacy of novel treatments in psychiatric patients based on animal models, this particular model has shown robust validity as an animal model of panic attacks. This preclinical model involves chronic inhibition of GABA synthesis (with L-AG, a glutamic acid decarboxylase inhibitor) in the DMH/PeF, a key panic-generating brain region (Shekhar, 1994). This produces ‘panic-prone’ rats that are highly anxious and exhibit panic-like cardio-respiratory responses to i.v. NaLac infusions similar to
patients with panic disorder (Johnson & Shekhar, 2006; Johnson et al. 2008b; Shekhar & Keim, 1997, 2000; Shekhar et al. 1996, 2006). The model has established robust face validity (Johnson & Shekhar, 2006; Johnson et al. 2008b; Shekhar & Keim, 1997, 2000; Shekhar et al. 1996, 2006). It has also demonstrated excellent predictive validity both for panic-inducing agents that elicit panic attacks in patients with panic disorder [e.g., NaLac, yohimbine, and inhalations of CO₂ (Shekhar & Keim, 1997)], and for anti-panic drugs, including known therapeutic agents such as benzodiazepine and antidepressants (Shekhar, 1994; Shekhar & Keim, 2000), as well as emerging novel therapies such as group II metabotropic glutamate agonists (Shekhar & Keim, 2000). Finally, in a series of preclinical studies, that also used this animal model of panic vulnerability, a novel translocator protein agonist (which enhances the central inhibitory effects of GABA) was effective in blocking NaLac-induced panic-like responses, which in follow-up clinical trials also showed anti-panic properties, further strengthening its predictive validity (Rupprecht et al. 2009). The construct validity of this model is supported by the fact that neural circuits of the DMH/PeF regulate behavioural and autonomic components of the ‘fight-or-flight’ response in rats (Andreatini et al. 2001; DiMicco et al. 2002), and are implicated in eliciting panic-like responses in humans (Boshuisen et al. 2002) and animals (Johnson et al. 2008b). Furthermore, panic disorder patients have deficits in central GABA activity (Goddard et al. 2001) and pharmacological restoration of central GABA activity prevents panic attacks (Goddard et al. 2004), in agreement with prediction from this model.

Good clinical safety profile has recently been demonstrated for CRF₁ antagonists in preliminary clinical studies in patients with major depression. In subjects with major depression, CRF₁ antagonists appear to demonstrate some preliminary clinical efficacy in improving sleep (Held et al. 2004) and possibly other symptoms of depression (Zobel et al. 2000) without disrupting normal stress hormone response or having other metabolic side-effects (Kunzel et al. 2003, 2005). In the only published study of CRF₁ antagonists in subjects with an anxiety disorder, utilizing subjects with chronic generalized anxiety, CRF₁ antagonist failed to demonstrate clinical efficacy in contrast to a serotonin reuptake inhibitor which was the active comparator (Coric et al. 2010), suggesting that CRF₁ antagonists may not be effective in persistent, chronic anxiety states such as generalized anxiety disorder without acute episode of stress such as major depression or episodic stressful events such as panic attacks. Therefore, CRF₁ antagonists, like benzodiazepines, which show robust anti-anxiety and anti-panic effects with both acute treatments, may be ideal drugs for acutely blocking a panic attack. Such a stress load-dependent effect of CRF₁ antagonists is consistent with previous data showing that CRF₁ antagonists do not have an effect on the HPA axis (Keck et al. 2001; Steckler et al. 2006) or most behavioural tests of anxiety related behaviour (Keck et al. 2001; Steckler et al. 2006) in the absence of a stressor.

The exact mechanisms by which CRF₁ antagonists may elicit anti-panic effects are still unknown, and several possible pathways can be considered. As previously noted by Johnson et al. (2008b), NaLac-induced panic response is associated with robust activation of the central nucleus of the amygdala in this model. Similar activation of CRF-positive neurons of the central nucleus of the amygdala was also noted by Choi et al. (2005) utilizing the doxapram model of panic induction. Thus, the amygdalar CRF neuronal activation may be a common mechanism associated with a panic-like response and CRF₁ receptor antagonists could blunt the effects of CRF released. Similarly, amygdala CRF neurons also project to the brain stem and activate the locus coeruleus and dorsal raphe neurons, other key panic-generating systems which show selective responses in this panic model (Johnson et al. 2008a, b). A CRF₁ antagonist could potentially reduce panic response by thus reducing norepinephrine and serotonin release. Finally, we have strong evidence that panic attacks may be associated with hyperactive orexin neurons that are exclusive to the DMH/PeF and lateral hypothalamus (de Lecea et al. 1998; Sakurai, 2007); are known to regulate wakefulness and vigilance (Sakurai, 2007); and are excited by CRF and CRF₁ receptor agonists (Winsky-Sommerer et al. 2004). Therefore, blockade of the CRF₁ receptor may blunt a multitude of acute panic-generating systems and induce a robust anti-panic effect.

In conclusion, the present data suggest that selective CRF₁ receptor antagonists could be a novel target for developing anti-panic drugs that are as effective as benzodiazepines in acute treatment without their deleterious side-effects such as sedation, cognitive impairment and dependence.

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