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ARTICLE

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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 (CRF_1) 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 CRF_1 receptor antagonist prior to NaLac infusions blocked all NaLac-induced behaviour and cardiovascular responses. These data suggest that selective CRF_1 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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Key words: Anxiety, CRF, CRH, hypothalamus, lactate, panic.

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), CO_2 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

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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, CRF_1 and CRF_2, 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 & Berridge, 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 CRF_1 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, CRF_1 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 CRF_1 receptor antagonist (Steckler *et al.* 2006) utilizing a well characterized animal model of NaLac-induced panic-like response (Johnson & Shekhar, 2006; Johnson *et al.* 2008*b*; 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.* 2008*b*; Shekhar & Keim, 1997; Shekhar *et al.* 1996, 2006).

Intraperitoneal (i.p.) injections of JNJ19567470/CRA5626, a selective CRF_1 receptor antagonist

Five days following L-AG infusion onset, in a counterbalanced design, rats received an i.p. injection of either CRF_1 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_1 receptors expressed in brain tissue and cells that does not appear to be species specific; (2) acts as a CRF_1 receptor antagonist in AtT20 cells *in vitro*; (3) dose-dependently occupies CRF_1 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 M 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 × 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 $\geqslant$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-excitatory

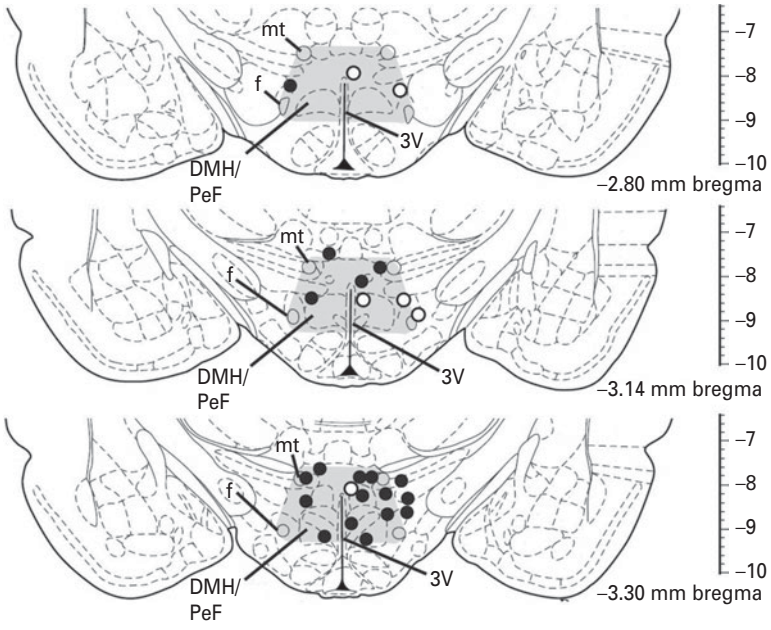

Fig. 1. Schematic representation of the infusion sites of L-allylglycine (L-AG; a GABA synthesis inhibitor) as determined by histology. Infusion cannula placements for L-AG infusions are illustrated as solid circles (●) for CRF_1 receptor antagonist experiments and open circles (○) for alprazolam experiments. 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.

(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 CRF_1 antagonist on behavioural and cardiovascular responses in L-AG-treated rats following NaLac challenge

Intraperitoneal injections of the highest dose of JNJ19567470/CRA5626 CRF_1 receptor antagonist 30 min prior to the lactate challenge attenuated lactate-induced 'anxiety', i.e. reduced social interaction time during the 5-min test ($F_{4,47}=6.5$, $p=0.000$; Fig. 2*a*), and changes (from baseline) in HR (treatment x time effect: $F_{57,589}=1.4$, $p=0.037$; Fig. 2*b*), and MAP (treatment × time effect: $F_{57,589}=2.4$, $p=0.000$; Fig. 2*d*) 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 ($F_{7,48}=1.0$, $p=0.413$) was noted over 5-min baseline +15 min of i.v. infusions in rats receiving the highest dose (40 mg/kg) of CRF_1 receptor antagonist. Although no increases in locomotor activity were noted in the 10-mg and 40-mg doses of CRF_1 receptor antagonist, a significant increase in locomotor activity was noted in the 3-mg/kg dose of CRF_1 receptor antagonist that was of longer duration than vehicle controls (see * symbols in Fig. 2*c* 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 ($F_{3,31}=0.6$, $p=0.595$), HR ($F_{3,31}=1.4$, $p=0.246$) or MAP ($F_{3,31}=0.8$, $p=0.508$) between groups (see legends to figures with baseline activity, HR and MAP ± S.E.M.). Nor did any dose of the CRF_1 receptor antagonist have

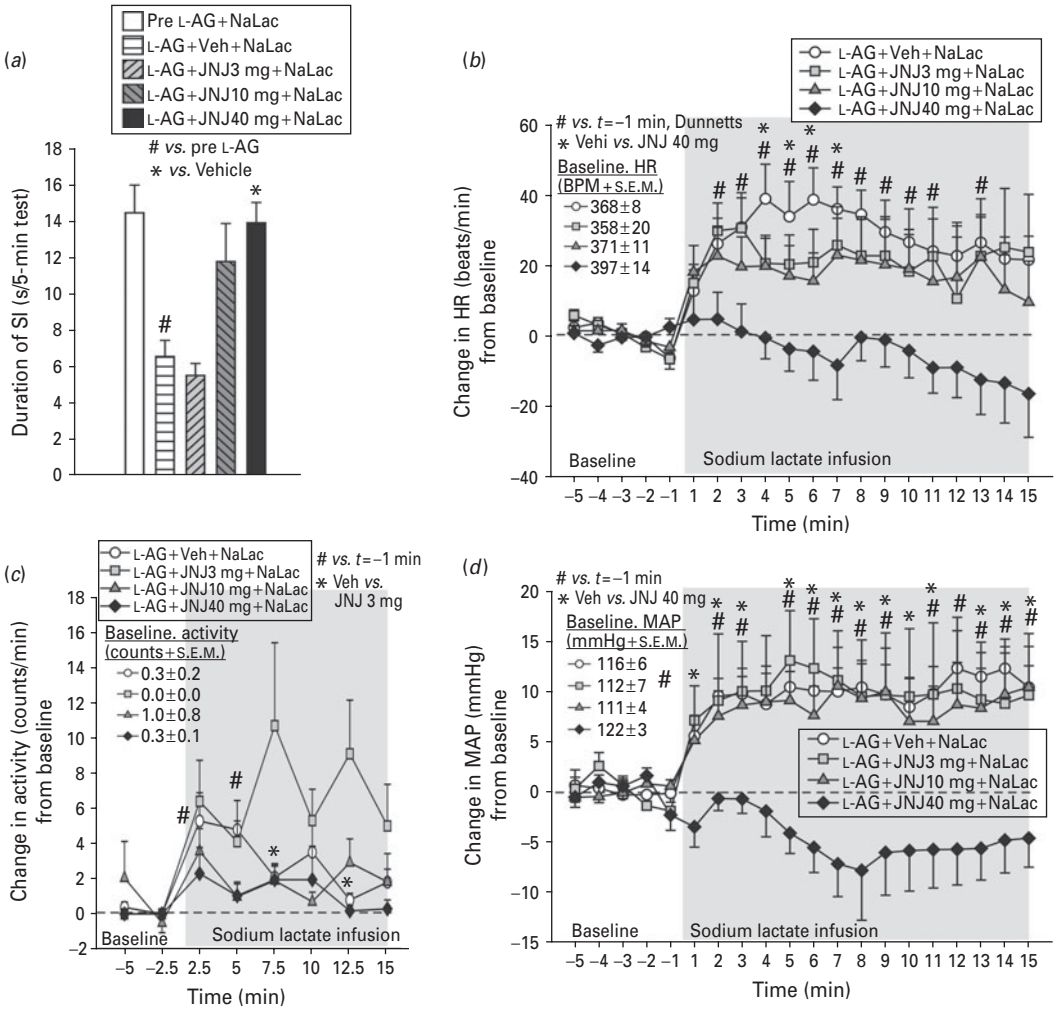

Fig. 2. Systematic (intraperitoneal) injection of the highest dose of CRF_1 receptor antagonist 30 min prior to lactate challenge attenuates lactate-induced (*a*) anxiety (and reduced social interaction time during the 5-min test), (*c*) 'flight'- (increased general activity) associated behaviour and changes (from 5-min baseline) in (*b*) tachycardia, and (*d*) pressor responses in rats made panic-prone with chronic reductions of GABA synthesis in the DMH/PeF [i.e. local infusions of L-allylglycine (L-AG), a GAD enzyme inhibitor]. The grey shaded area represents duration of intravenous NaLac infusions. The * symbol indicates between-subject differences between the 3 mg [(*c*) locomotor activity)] or 40 mg [(*b*) heart rate, and (*d*) blood pressure] CRF_1 receptor antagonist drug group and vehicle group using Tukey's HSD *post-hoc* test protected by ANOVA. The # symbol indicates within-subject time-points in the vehicle + L-AG + lactate group that are significantly different than the −1 min pre-lactate infusion time-point using one-way Dunnet's *post-hoc* test protected by one-way ANOVA analysing within-subject time effects. There was no significant baseline activity between groups [see panels (*b–d*) for baseline values for each treatment group for activity, heart rate (HR) and mean arterial blood pressure (MAP) ±S.E.M.].

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. 3*a*: HR (3 mg, $F_{19,60}=0.4$, $p=0.992$; 10 mg, $F_{19,180}=1.5$, $p=0.091$; 40 mg, $F_{19,120}=0.3$, $p=0.999$); Fig. 3*b*: MAP (3 mg, $F_{19,60}=0.2$, $p=1.000$; 10 mg, $F_{19,180}=0.9$, $p=0.595$; 40 mg, $F_{19,120}=0.8$, $p=0.640$); Fig. 3*c*: activity (3 mg, $F_{19,60}=0.6$, $p=0.850$; 10 mg, $F_{19,180}=0.7$, $p=0.814$; 40 mg, $F_{19,120}=0.8$, $p=0.640$)]. There were no differences in $t-20$ min to $t-15$ min baseline in HR ($F_{3,29}=0.2$, $p=0.905$) or MAP ($F_{3,29}=0.7$, $p=0.553$), but activity was higher at baseline in the 3-mg group, compared to vehicle group ($F_{3,29}=4.4$, $p=0.011$). Two telemetry probes malfunctioned due to low batteries in the l-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 l-AG + vehicle group (i.e. $n=13$).

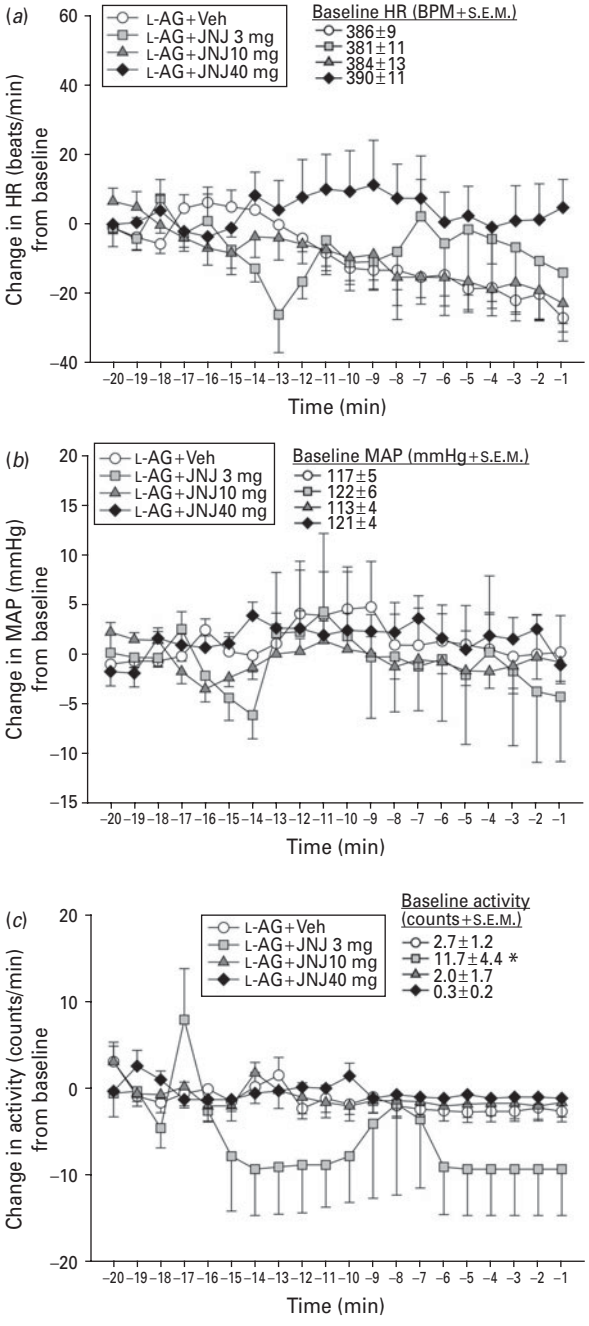

Fig. 3. Systematic (intraperitoneal) injection of three separate doses of CRF_1 receptor antagonist did not alter (*a*) 'flight' – (increased general activity) associated behaviour, (*b*) tachycardia, and (*c*) pressor responses in L-AG administered over time prior to sodium lactate (NaLac) challenge (between −20 min to onset of NaLac challenge). A difference between baseline activity of the 3-mg and vehicle group was noted [see * in panel (*c*)]. There were no other significant differences between baseline activity between groups for heart rate (HR) or mean arterial blood pressure (MAP) [see panels (*a*–*c*) for baseline values for each treatment group for activity, HR and MAP ± S.E.M.].

Effects of a prior treatment with alprazolam on behavioural and cardiovascular responses in L-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 ($F_{2,15} = 19.9$, $p = 0.000$; Fig. 4*a*) and changes (from baseline) in HR (treatment × time effect: $F_{19,190} = 2.0$, $p = 0.011$; and a within-subjects increase in HR post-i.v. infusion in L-AG + vehicle group (time effect: $F_{19,100} =$, $p = 0.11$; Fig. 4*b*) 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 L-AG + vehicle group ($F_{19,100} = 3.3$, $p < 0.001$), therefore a non-parametric Kruskal–Wallis *post-hoc* test was used to compare pre-i.v. infusion HR ($t-1$ m) to each post-i.v. infusion HR. Panic-prone rats displayed no change in general activity (treatment × time effect: $F_{7,70} = 0.6$, $p = 0.737$; Fig. 4*c*) or MAP (treatment × time effect: $F_{19,190} = 0.4$, $p = 0.987$; Fig. 4*d*) following NaLac. There was no significant differences in baseline activity ($t_5 = -0.2$, $p = 0.853$), HR ($t_5 = -0.3$, $p = 0.770$) or MAP ($t_5 = -0.04$, $p = 0.970$) in any rat prior to NaLac infusion (see figure legends with baseline activity, HR and MAP ± 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 cardio-excitatory responses, is potentially indicative of flight-associated panic behaviour. Overall, this suggests that the 3 mg/kg dose was

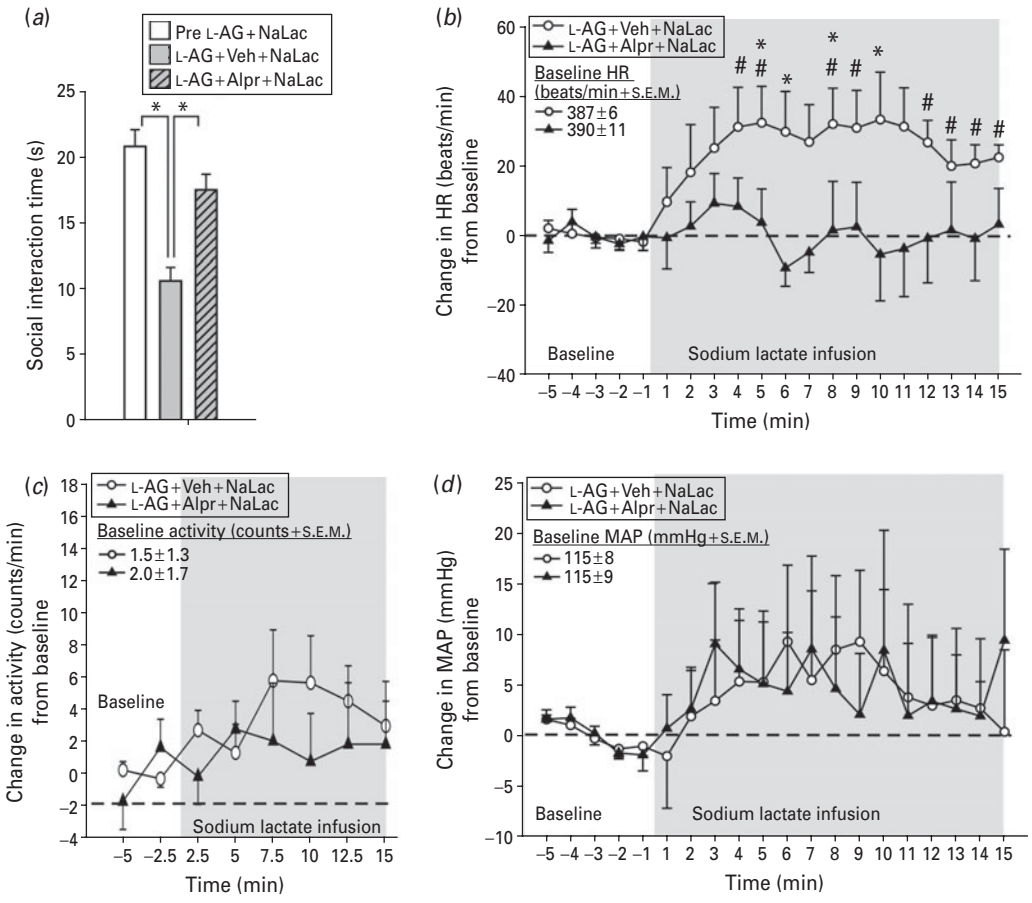

Fig. 4. Systematic (intraperitoneal) injection of alprazolam 30 min prior to lactate challenge attenuates lactate-induced (*a*) anxiety (and reduced social interaction time during the 5-min test) associated behaviour and changes (from 5 min baseline) in (*b*) tachycardia responses in rats made panic-prone with chronic reductions of GABA synthesis in the DMH/PeF [i.e. local infusions of L-allylglycine (L-AG), a GAD enzyme inhibitor]. Panic-prone rats did not display changes in (*c*) general activity or (*d*) mean arterial blood pressure following sodium lactate (NaLac). The grey shaded area represents duration of intravenous NaLac infusions. The * symbol indicates between-subject differences (vehicle *vs.* alprazolam) using Tukey's HSD *post-hoc* test protected by ANOVA. The # symbol indicates within-subject time-points in the vehicle + L-AG + lactate group that are significantly different from the −1 min pre-lactate infusion time-point using a Kruskal–Wallis test protected by one-way ANOVA analysing within-subject time effects. There was no significant baseline activity between groups [see panels (*b–d*) for baseline values for each treatment group for activity, heart rate (HR) and mean arterial blood pressure (MAP) ± S.E.M.].

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 cardiorespiratory responses to i.v. NaLac infusions similar to

patients with panic disorder (Johnson & Shekhar, 2006; Johnson *et al.* 2008*b*; Shekhar & Keim, 1997, 2000; Shekhar *et al.* 1996, 2006). The model has established robust face validity (Johnson & Shekhar, 2006; Johnson *et al.* 2008*b*; 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 CO_2 (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.* 2008*b*). 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 CRF_1 antagonists in preliminary clinical studies in patients with major depression. In subjects with major depression, CRF_1 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 CRF_1 antagonists in subjects with an anxiety disorder, utilizing subjects with chronic generalized anxiety, CRF_1 antagonist failed to demonstrate clinical efficacy in contrast to a serotonin reuptake inhibitor which was the active comparator (Coric *et al.* 2010), suggesting that CRF_1 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, CRF_1 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 CRF_1 antagonists is consistent with previous data showing that CRF_1 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 CRF_1 antagonists may elicit anti-panic effects are still unknown, and several possible pathways can be considered. As previously noted by Johnson *et al.* (2008*b*), 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 CRF_1 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.* 2008*a*, *b*). A CRF_1 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 CRF_1 receptor agonists (Winsky-Sommerer *et al.* 2004). Therefore, blockade of the CRF_1 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 CRF_1 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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References

Abshire VM, Hankins KD, Roehr KE, DiMicco JA (1988). Injection of L-allylglycine into the posterior hypothalamus in rats causes decreases in local GABA which correlate with increases in heart rate. *Neuropharmacology* **27**, 1171–1177.

Andreatini R, Blanchard C, Blanchard R, Brandao ML, *et al.* (2001). The brain decade in debate: II. Panic or anxiety? From animal models to a neurobiological basis. *Brazilian Journal of Medical and Biological Research* **34**, 145–154.

APA (1994). *Diagnostic and Statistical Manual of Mental Disorders* – 4th edn (DSM-IV). Washington, DC: American Psychiatric Association.

Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, *et al.* (2005). Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. *Journal of Psychopharmacology* **19**, 567–596.

Baldwin HA, Rassnick S, Rivier J, Koob GF, *et al.* (1991). CRF antagonist reverses the 'anxiogenic' response to ethanol withdrawal in the rat. *Psychopharmacology* (*Berlin*) **103**, 227–232.

Bandelow B, Zohar J, Hollander E, Kasper S, *et al.* (2008). World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders – first revision. *World Journal of Biological Psychiatry* **9**, 248–312.

Boshuisen ML, Ter Horst GJ, Paans AM, Reinders AA, *et al.* (2002). rCBF differences between panic disorder patients and control subjects during anticipatory anxiety and rest. *Biological Psychiatry* **52**, 126–135.

Britton DR, Koob GF, Rivier J, Vale W (1982). Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sciences* **31**, 363–367.

Britton KT, Morgan J, Rivier J, Vale W, *et al.* (1985). Chlordiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test. *Psychopharmacology* **86**, 170–174.

Brown MR, Hauger R, Fisher LA (1988). Autonomic and cardiovascular effects of corticotropin-releasing factor in the spontaneously hypertensive rat. *Brain Research* **441**, 33–40.

Chalmers DT, Lovenberg TW, De Souza EB (1995). Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *Journal of Neuroscience* **15**, 6340–6350.

Choi SH, Kim SJ, Park SH, Moon BH, *et al.* (2005). Doxapram increases corticotropin-releasing factor immunoreactivity and mRNA expression in the rat central nucleus of the amygdala. *Peptides* **26**, 2246–2251.

Cloos JM, Ferreira V (2009). Current use of benzodiazepines in anxiety disorders. *Current Opinion in Psychiatry* **22**, 90–95.

Cole BJ, Koob GF (1988). Propranolol antagonizes the enhanced conditioned fear produced by corticotropin releasing factor. *Journal of Pharmacology and Experimental Therapeutics* **247**, 902–910.

Coric V, Feldman HH, Oren DA, Shekhar A, *et al.* (2010). Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. *Depression and Anxiety* **27**, 417–425.

Dautzenberg FM, Hauger RL (2002). The CRF peptide family and their receptors: yet more partners discovered. *Trends in Pharmacological Sciences* **23**, 71–77.

de Lecea L, Kilduff TS, Peyron C, Gao X, *et al.* (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Sciences USA* **95**, 322–327.

De Souza EB, Insel TR, Perrin MH, Rivier J, *et al.* (1985). Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. *Journal of Neuroscience* **5**, 3189–3203.

DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV (2002). The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. *Pharmacology Biochemistry and Behavior* **71**, 469–480.

Dunn AJ, Berridge CW (1987). Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems. *Pharmacology Biochemistry and Behavior* **27**, 685–691.

Dunn AJ, File SE (1987). Corticotropin-releasing factor has an anxiogenic action in the social interaction test. *Hormones & Behavior* **21**, 193–202.

File SE (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *Journal of Neuroscience Methods* **2**, 219–238.

Gehlert DR, Shekhar A, Morin SM, Hipskind PA, *et al.* (2005). Stress and central Urocortin increase anxiety-like behavior in the social interaction test via the CRF1 receptor. *European Journal of Pharmacology* **509**, 145–153.

Goddard AW, Mason GF, Almai A, Rothman DL, *et al.* (2001). Reductions in occipital cortex GABA levels in panic disorder detected with 1 h-magnetic resonance spectroscopy. *Archives of General Psychiatry* **58**, 556–561.

Goddard AW, Mason GF, Appel M, Rothman DL, *et al.* (2004). Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. *American Journal of Psychiatry* **161**, 2186–2193.

Gorman JM, Papp LA, Coplan JD, Martinez JM, *et al.* (1994). Anxiogenic effects of CO2 and hyperventilation in patients

with panic disorder. *American Journal of Psychiatry* **151**, 547–553.

Gutman DA, Coplan J, Papp L, Martinez J, Gorman J (2005). Doxapram-induced panic attacks and cortisol elevation. *Psychiatry Research* **133**, 253–261.

Held K, Kunzel H, Ising M, Schmid DA, *et al.* (2004). Treatment with the CRH1-receptor-antagonist R121919 improves sleep-EEG in patients with depression. *Journal of Psychiatric Research* **38**, 129–136.

Hokfelt T, Bartfai T, Bloom F (2003). Neuropeptides: opportunities for drug discovery. *Lancet Neurology* **2**, 463–472.

Johnson P, Lowry C, Truitt W, Shekhar A (2008*a*). Disruption of GABAergic tone in the dorsomedial hypothalamus attenuates responses in a subset of serotonergic neurons in the dorsal raphe nucleus following lactate-induced panic. *Journal of Psychopharmacology* **22**, 642–652.

Johnson PL, Shekhar A (2006). Panic-prone state induced in rats with GABA dysfunction in the dorsomedial hypothalamus is mediated by NMDA receptors. *Journal of Neuroscience* **26**, 7093–7104.

Johnson PL, Truitt WA, Fitz SD, Lowry CA, *et al.* (2008*b*). Neural pathways underlying lactate-induced panic. *Neuropsychopharmacology* **33**, 2093–2107.

Keck ME, Kern N, Erhardt A, Unschuld PG, *et al.* (2008). Combined effects of exonic polymorphisms in CRHR1 and AVPR1B genes in a case/control study for panic disorder. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics* **147B**, 1196–1204.

Keck ME, Welt T, Wigger A, Renner U, *et al.* (2001). The anxiolytic effect of the CRH(1) receptor antagonist R121919 depends on innate emotionality in rats. *European Journal of Neuroscience* **13**, 373–380.

Keegan CE, Herman JP, Karolyi IJ, O'Shea KS, *et al.* (1994). Differential expression of corticotropin-releasing hormone in developing mouse embryos and adult brain. *Endocrinology* **134**, 2547–2555.

Keller C, Bruelisauer A, Lemaire M, Enz A (2002). Brain pharmacokinetics of a nonpeptidic corticotropin-releasing factor receptor antagonist. *Drug Metabolism and Disposition* **30**, 173–176.

Ku YH, Tan L, Li LS, Ding X (1998). Role of corticotropin-releasing factor and substance P in pressor responses of nuclei controlling emotion and stress. *Peptides* **19**, 677–682.

Kunzel HE, Ising M, Zobel AW, Nickel T, *et al.* (2005). Treatment with a CRH-1-receptor antagonist (R121919) does not affect weight or plasma leptin concentration in patients with major depression. *Journal of Psychiatric Research* **39**, 173–177.

Kunzel HE, Zobel AW, Nickel T, Ackl N, *et al.* (2003). Treatment of depression with the CRH-1-receptor antagonist R121919: endocrine changes and side effects. *Journal of Psychiatric Research* **37**, 525–533.

Liang KC, Lee EH (1988). Intra-amygdala injections of corticotropin releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats. *Psychopharmacology* (*Berlin*) **96**, 232–236.

Liang KC, Melia KR, Miserendino MJ, Falls WA, *et al.* (1992). Corticotropin-releasing factor: long-lasting facilitation of the acoustic startle reflex. *Journal of Neuroscience* **12**, 2303–2312.

Liebowitz MR, Fyer AJ, Gorman JM, Dillon D, *et al.* (1984). Lactate provocation of panic attacks. I. Clinical and behavioral findings. *Archives of General Psychiatry* **41**, 764–770.

Liebowitz MR, Gorman JM, Fyer A, Dillon D, *et al.* (1986). Possible mechanisms for lactate's induction of panic. *American Journal of Psychiatry* **143**, 495–502.

Liebowitz MR, Gorman JM, Fyer AJ, Levitt M, *et al.* (1985). Lactate provocation of panic attacks. II. Biochemical and physiological findings. *Archives of General Psychiatry* **42**, 709–719.

Nutt DJ, Ballenger JC, Sheehan D, Wittchen HU (2002). Generalized anxiety disorder: comorbidity, comparative biology and treatment. *International Journal of Neuropsychopharmacology* **5**, 315–325.

Paxinos G, Watson C (1997). *The Rat Brain Stereotaxic Coordinates*. San Diego: Academic Press.

Perrin MH, Vale WW (1999). Corticotropin releasing factor receptors and their ligand family. *Annals of the New York Academy of Sciences* **885**, 312–328.

Risbrough VB, Stein MB (2006). Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. *Hormones and Behavior* **50**, 550–561.

Rupprecht R, Rammes G, Eser D, Baghai TC, *et al.* (2009). Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science* **325**, 490–493.

Sakurai T (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nature Review Neuroscience* **8**, 171–181.

Samuels BC, Zaretsky DV, DiMicco JA (2004). Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* **287**, R472–R478.

Sanders SK, Shekhar A (1995). Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacology Biochemistry and Behavior* **52**, 701–706.

Shekhar A (1993). GABA receptors in the region of the dorsomedial hypothalamus of rats regulate anxiety in the elevated plus-maze test. I. Behavioral measures. *Brain Research* **627**, 9–16.

Shekhar A (1994). Effects of treatment with imipramine and clonazepam on an animal model of panic disorder. *Biological Psychiatry* **36**, 748–758.

Shekhar A, DiMicco JA (1987). Defense reaction elicited by injection of GABA antagonists and synthesis inhibitors into the posterior hypothalamus in rats. *Neuropharmacology* **26**, 407–417.

Shekhar A, Johnson PL, Sajdyk TJ, Fitz SD, *et al.* (2006). Angiotensin-II is a putative neurotransmitter in lactate-induced panic-like responses in rats with disruption of GABAergic inhibition in the dorsomedial hypothalamus. *Journal of Neuroscience* **26**, 9205–9215.

Shekhar A, Katner JS (1995). Dorsomedial hypothalamic GABA regulates anxiety in the social interaction test. *Pharmacology Biochemistry and Behavior* **50**, 253–258.

Shekhar A, Keim SR (1997). The circumventricular organs form a potential neural pathway for lactate sensitivity: implications for panic disorder. *Journal of Neuroscience* **17**, 9726–9735.

Shekhar A, Keim SR (2000). LY354740, a potent group II metabotropic glutamate receptor agonist prevents lactate-induced panic-like response in panic-prone rats. *Neuropharmacology* **39**, 1139–1146.

Shekhar A, Keim SR, Simon JR, McBride WJ (1996). Dorsomedial hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions. *Pharmacology Biochemistry and Behavior* **55**, 249–256.

Steckler T, Nakazato A, Kennis L, Mackie C, *et al.* (2006). CRF1 antagonists – therapeutic implications for affective and mood disorders. *Société de Chimie Thérapeutique* **32**, 1–19.

Swanson S, Simmons DM (1989). Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *Journal of Comparative Neurology* **285**, 413–435.

Swerdlow NR, Geyer MA, Vale WW, Koob GF (1986). Corticotropin-releasing factor potentiates acoustic startle in rats: blockade by chlordiazepoxide. *Psychopharmacology* **88**, 147–152.

Takahashi LK, Kalin NH, Vanden Burgt JA, Sherman JE (1989). Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behavioral Neuroscience* **103**, 648–654.

Vale W, Spiess J, Rivier C, Rivier J (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **213**, 1394–1397.

Vickers K, McNally RJ (2005). Respiratory symptoms and panic in the National Comorbidity Survey: a test of Klein's suffocation false alarm theory. *Behavior Research and Therapy* **43**, 1011–1018.

Winsky-Sommerer R, Yamanaka A, Diano S, Borok E, *et al.* (2004). Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *Journal of Neuroscience* **24**, 11439–11448.

Zobel AW, Nickel T, Kunzel HE, Ackl N, *et al.* (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *Journal of Psychiatric Research* **34**, 171–181.