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Preliminary evidence of anxiolytic effects of the CRF_1 receptor antagonist R317573 in the 7.5% CO_2 proof-of-concept experimental model of human anxiety

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Abstract

We have validated the use of prolonged inhalation of 7.5% carbon dioxide (CO_2) as a human model of anxiety and have shown that drugs from two prototypical classes of anxiolytics, benzodiazepines and a serotonin reuptake inhibitor, attenuate CO_2-induced symptoms (Bailey et al., 2007a). Preclinical evidence suggests that drugs acting at the corticotropin-releasing factor (CRF) system may be useful for the treatment of depression, anxiety, and other stress-related disorders (Valdez, 2006), hence we have now examined the effects of a CRF_1 receptor antagonist in the 7.5% CO_2 model. In a randomized double-blind, placebo-controlled, study in 32 healthy participants we examined the effects of 7 days of treatment with the CRF_1 receptor antagonist, R317573, at a dose that shows a favourable safety profile and is comparable with those effective in preclinical models (40 mg). On day 8, eight of the placebo-treated group received lorazepam (LZP) 2 mg as a positive control. All participants underwent 20 min inhalation of 7.5% CO_2-enriched air. Subjective reports of peak gas effects were assessed using visual analogue scales and questionnaires. The mean age of participants was 26 years, and 13 were male. The peak effects of CO_2 were expressed as a difference from baseline scores obtained while breathing air alone. Compared with placebo (PLAC), both drug groups showed a decrease in all subjective symptoms, total score on the panic symptom inventory (CRF 11 [2.6], PLAC 16.4 [3.1], LZP 2.9 [3.0]) and a generalized anxiety disorder symptom scale (CRF 2.2 [1.5], PLAC 8.2 [2.2], LZP 1.1 [1.5]). We have shown that a drug that acts to inhibit the CRF_1 receptor shows efficacy in the 7.5% CO_2 model of anxiety in healthy participants.

Keywords

Anxiety, CO_2 inhalation, CRF receptor antagonist, proof-of-concept model, translation

Introduction

We have been developing two human models of anxiety using the inhalation of carbon dioxide. One is the further development of the single vital capacity inhalation of 35% CO_2 which produces a model of acute, panic-like anxiety that is of brief duration and produces an increase in cortisol (Argyropoulos et al., 2002; Kaye et al., 2004; van Duinen et al., 2005). The other uses 7.5% CO_2 inhaled for 20 min, and this leads to symptoms more like those seen with generalized anxiety disorder (GAD), such as increases in anxiety and worry and raised blood pressure, heart rate and respiratory rate (Bailey et al., 2005).

Our studies to date have shown that the subjective response to inhaling hypercapnic gas can be attenuated with anxiolytic medications, particularly those acting at the GABA/benzodiazepine receptor such as the anxiolytics lorazepam and alprazolam and the hypnotic zolpidem (Bailey et al., 2007a, 2009), but also a selective serotonin reuptake inhibitor, paroxetine (Bailey et al., 2007a). We have also shown that 7.5% CO_2 produces symptoms in patients with a diagnosis of GAD (Seddon et al., 2011). During this series of studies we have been developing and validating a proof-of-concept model for use in the early phase of drug development and have concluded from our studies, ongoing laboratory studies (unpublished) and the published literature, that the inhalation of CO_2 is a valid trans-species, translational model of anxiety (see Bailey and

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Nutt, 2008; Bailey et al., 2011). This report demonstrates this concept in action using a novel compound that shows a good anxiolytic profile in animal models.

Preclinical and some clinical evidence suggests that drugs that act at the corticotrophin-releasing factor (CRF) system may be useful for the treatment of psychiatric disorders, such as anxiety and depression or other stress-related disorders (Valdez, 2006). CRF is a 41 amino acid peptide produced by the paraventricular nucleus (PVN). CRF is widely distributed through the central nervous system (CNS), with the highest concentrations found in the hypothalamus. Two G-protein-linked CRF receptors have been identified, CRF_1 and CRF_2, with different central localization. In rats, CRF_1 is mostly present in the cortex, limbic system, brainstem and cerebellum, whilst CRF_2 appears to be mainly localized in subcortical structures, especially the hypothalamus (Chalmers et al., 1995). Due to this pattern of distribution, it has been postulated that CRF may be involved in stress-related cognition, emotion, stress and autonomic function (De Souza and Grigoriadis, 1995). During acute stress there is the release of CRF in many parts of the brain. When it originates from the PVN it leads to adrenocorticotropic hormone (ACTH) release, which in turn promotes cortisol production and release.

This wide range of stress-related effects of CRF has led to the development of CRF receptor-targeted drug treatments, with most of the evidence to date being focused on the antagonism of the CRF_1 receptor. These antagonists produce anxiolytic and antidepressant effects in animal models (for reviews see Ising and Holsboer, 2007; Risbrough and Stein, 2006). There is also emerging evidence of efficacy in clinical studies, particularly in the treatment of anxiety and depression (Ising and Holsboer, 2007), although this evidence is not consistent, with a recent report showing lack of efficacy of a different antagonist in major depression (Binneman et al., 2008) and more recently in GAD (Coric et al., 2010). Further evidence of the use of CRF_1 receptor antagonists in stress-related disorders is shown in a study using the Trier Social Stress test. In this study the hormonal response was attenuated in the treatment group compared with placebo, whilst the normal diurnal secretion of CRF was not changed (Ising et al., 2007).

In view of this evidence, we proposed a proof-of-concept study to test the efficacy of a novel CRF_1 receptor antagonist, R317573, in the 7.5% CO_2 model of anxiety in healthy participants. A proof-of-concept model is attractive since it can be introduced into the early phases of a drug development programme prior to initiating treatment studies in patients, which can take a considerable time to complete (e.g. Coric et al., 2010). We therefore conducted a double-blind, placebo-controlled study, with positive control, in healthy participants. The primary objective of this study was to determine whether 7 days of treatment with the CRF antagonist could reduce CO_2-induced symptoms of anxiety when compared with placebo. In addition to subjective measures, we incorporated physiological and biological markers of response.

R317573 is an orally active, non-peptidergic, potent and highly selective antagonist at the CRF_1 receptor. It has good brain penetration and good in vitro and metabolic stability. In binding studies in various cells and tissues, R317573 had high selectivity for the CRF_1 receptor over the CRF_2 receptor. The compound has shown antidepressant-like effects in some, but not all, animal models of depression, and has shown significant anxiolytic-like effects in a battery of tests measuring anxiety-related behaviour (Steckler et al., 2006). More recently, in healthy male subjects, R317573 has dose-dependently produced acute changes in regional cerebral glucose metabolism in regions that may be behaviourally relevant to mood and anxiety disorders (Schmidt et al., 2010).

Pharmacokinetic studies in humans show rapid absorption into plasma, with a biphasic elimination period and elimination half-life of approximately 20 h. The dose selected for this study is representative of the doses used in animal models to show anxiolytic effects, and displayed a good safety profile in phase I studies in man. This study represents a translational approach to drug development and will determine whether R317573 has an anxiolytic-like effect in a human model of anxiety.

Methods

Ethical considerations

The study was performed in accordance with ICH Good Clinical Practice, with approval from an Independent Research Ethics Committee (Plymouth Phase I Clinical Trials Independent Ethics Committee), Bath Research Ethics Committee for site-specific assessment and relevant Health Service Trusts regulatory approval (United Bristol Healthcare Trust and Avon and Wiltshire Mental Health Partnership Trust). The study sponsor was Johnson and Johnson Pharmaceutical Research and Development, and administrative support was provided by P1Vital Ltd.

Research participants

All participants were recruited either from our volunteer database, by advertisements on University and United Bristol Healthcare Trust property, or via local media publications, as approved by the Ethics Committee. Each participant gave written informed consent after one of the investigators explained the nature, purpose and risks of the study, and prior to any study-related procedures. All participants were screened with a medical and psychiatric history, physical examination, electrocardiogram, haematological and biochemical blood testing and urine test for detecting drugs of abuse, and pregnancy for females. All were interviewed by a physician or psychiatrist and underwent screening using the Neuropsychiatric Interview MINI shortform, (developed and validated by Sheehan et al., University of South Florida and Lecrubier et al., Paris) to ensure they, or their first-degree relatives, did not have a history of anxiety or panic disorder, or other mental illness. Other exclusion criteria were: current or history of drug or alcohol abuse or dependence; smoking more than six cigarettes per day; current or history of cardiovascular, respiratory or renal disease, hypertension, migraine, and epilepsy. Neither concomitant medications nor intake of any medication (apart from occasional aspirin or paracetamol or the oral contraceptive pill) were allowed for 8 weeks prior to and during study period. The family doctor of the participant was contacted and a response confirming health status

was required prior to participation. All participants were compensated (£600) for their involvement.

Healthy participants, rather than patients with an anxiety disorder, were used in this experimental study. This was because this CO_2 test has been developed to avoid the requirement for anxiety patients to be recruited into phase 1 studies (see introduction). These studies are designed to look at an anxious response (both psychological and physiological) rather than the effects of a panic attack, which have been well documented.

Study design

The study was double-blind, randomized, placebo and active controlled. The study phases consisted of an eligibility screening (between 21 and 2 days prior to the first dose of study drug), a double-blind treatment phase (day 1 to day 8), a 7.5% CO_2 challenge test on day 8 and a follow-up examination at approximately 4–7 days after the last dose administration.

Participants were randomly assigned to receive either R317573 (40 mg) once daily in the evening on day 1–7 and placebo to lorazepam in the morning of day 8, or placebo to R317573 once daily in the evening on day 1–7 and placebo to lorazepam in the morning of day 8, or placebo to R317573 once daily in the evening on day 1–7 and lorazepam 2 mg in the morning of day 8. For clarity, see the dosing schedule in Table 1.

Screening

After the signed informed consent procedure, all potential participants underwent the screening examination. This consisted of medical and psychiatric history, a physical examination (including height, weight, and body temperature), supine and standing blood pressure and heart rate and a 12-lead electrocardiograph (ECG). A neuropsychiatric interview (the MINI) was completed to screen for current or past psychiatric disorder. Participants with a body mass index (BMI) outside of the range 18–28 kg/m^2 were excluded.

If potential participants were judged to be healthy after this initial screening, they underwent the inhalation of 7.5% CO_2 for 20 min to assess their reactivity to the gas. Reactivity was rated using the panic symptom inventory (PSI) just after the inhalation period, rated for peak effects. For the purposes of this study, as set out in the study protocol, an 'arbitrary' measure of significant response to the inhalation was defined as a total score $\geq$20 points in the PSI taken just after the inhalation to assess peak effect. If they did not meet this threshold, or decide not to inhale the gas on a second occasion, no further screening took place. This was done to ensure that participants were 'responders' to the anxiogenic effects of 7.5% of CO_2, and also so that all would experience the same anticipatory effects on the test day. This 'arbitrary' decision were made by the research team and study sponsor, and was based on observations from our unpublished database of healthy participant responses to the inhalation of 7.5% CO_2.

Table 1. Dosing schedule

Group	Day 1–7 evening dose	Day 8 morning dose
R317573 (40 mg)	R317573 (40 mg)	Placebo
Placebo	Placebo	Placebo
Lorazepam 2 mg	Placebo	Lorazepam 2 mg

For participants who met the criteria, a blood sample for clinical laboratory analysis (including serology and thyroid stimulating hormone (TSH)) was obtained. A urine sample was tested for drugs of abuse and – for females only – a pregnancy test, plus, for all subjects, an alcohol breath test was performed.

If all the relevant inclusion criteria were met, the participant was invited to join the study and booked in for a randomization visit, which was day 1. Each subsequent study day (day 2–7) took place under supervision in a general hospital clinical research facility. During the study period (Days 1–follow-up) participants carried an information card reminding them not to drink alcohol, take any other medication, drive or operate machinery and with 24-h emergency contact details. In addition, they were advised not to perform any strenuous exercise from 48 h prior to the first dose of medication until follow-up; not to take any foodstuffs containing poppy seeds, quinine, grapefruit or Seville oranges. They were asked to keep caffeine-containing drinks to a minimum, to use effective and adequate contraception measures (male and female) and to inform the research team of any changes in health.

For study days 2–7, arrival at the research unit was at approximately 19:00 when participants underwent a breath test to check that no recent alcohol had been drunk and were questioned about any concomitant medication. Adverse effects were elicited by the question 'have you experienced any changes in your health since your last visit?'. Participants were then given a standardized hot meal and dosed 15 min after completion of food. Participants then remained in the research unit until 22:00, when transport was provided home.

On night 5 of dosing, in addition to the above, participants provided a specimen of urine which was checked for drugs of abuse and, in females, for pregnancy. They also had an ECG recorded, blood pressure and heart rate measured and a blood sample taken for safety evaluations.

During the evening of night 7 subjects were cannulated, and a 10 ml blood sample was taken before dosing, and at 60, 90 and 120 min post-dosing, for pharmacokinetic measures. At 22:00 participants were admitted to an in-patient clinical research facility so that conditions could be standardized prior to the CO_2 challenge on the morning of day 8.

On awakening, a fasting blood sample was taken for safety analysis and a standardized breakfast was given. Participants were then escorted from the ward to the clinical research room in preparation for the CO_2 challenge.

CO_2 challenge on day 8

On arrival, adverse events and use of any concomitant medication were assessed and recorded. Study procedures and

timings were explained, and then a single oral dose containing either 2 mg lorazepam (for placebo group) or matching placebo (for placebo and R317573 group) was given. After 45 min, ECG, heart rate and blood pressure were recorded. Baseline visual analogue scale (VAS) ratings were recorded and pre-CO_2 challenge blood samples for stress hormones drawn. At 5 min prior to the first inhalation, VAS were repeated, subjective questionnaires completed and a blood sample for assessment of pharmacokinetic levels drawn.

Rating scales

Common to many of our previously reported CO_2 studies, we used our standard set of subjective rating scales to measure changes in subjective stage. The VAS consisted of a set of individual cards labelled along a 10-cm line with anchor points, on a scale of 0 (not at all) to 100 (the most ever) using the adjectives: fearful, relaxed, anxious, happy, feel like leaving, stressed, tense, nervous, irritable and worried. Participants were required to give a verbal numerical response. The subjective questionnaires used were the Spielberger State Anxiety Inventory (SSAI) (Spielberger, 1983); the PSI, which lists 34 anxiety-related items to be rated from 0 (not at all) to 4 (very severe); and the Generalized Anxiety Disorder Criteria Inventory (GAD-C), which is a 13-item list based on GAD diagnostic criteria, rated from 0 (absent) to 4 (very marked).

The 7.5% CO_2 challenge and assessment tools used have been fully described elsewhere (Bailey et al., 2005, 2007a), but briefly, the participant inhaled 20 min of piped air, had 20 min of no inhalation for assessments and then 20 min of 7.5% CO_2 in air. Both gases were inhaled via a nasal–oral face mask (Hans Rudolf), and the order of gas inhalation was single-blind. During both inhalation periods, continuous blood pressure and heart rate were measured using Finapres. Blood was drawn at 10-min intervals, from which the levels of stress hormones ACTH, AVP and cortisol were determined. Peak effects of inhalation were rated using VAS and subjective questionnaires. At the end of the study session, participants were allowed home if deemed fully recovered.

A follow-up visit occurred 4–7 days after the CO_2 challenge day. Physical health was assessed with examination, ECG and blood tests. Any continuing adverse events were documented and followed-up until resolved.

Drugs

Study medication was provided in prepared foil blister packs with study day labels attached. Randomization and drugs were provided by the study sponsor, Johnson and Johnson Pharmaceutical Research and Development Global Clinical Supply Unit. All doses were given on site under supervision. All participants were required to carry a card detailing emergency contact procedures. Treatments, consisting of two capsules, were taken orally with water 15 min after eating an evening meal, apart from day 8 when lorazepam or placebo was taken with water in the morning 90 min prior to the first gas administration.

Pharmacokinetics

Venous blood samples for assessment of plasma concentrations of R317573 and R337676, a carboxylic acid metabolite, were taken (4.0 ml into heparinized tubes), pre-dose and at 1, 1.5 and 2 h post dose on day 7, and on day 8 samples were taken before CO_2 and 30 and 60 min post-CO_2. R337676 was included after it had been identified as being the principal circulating plasma metabolite of R317573 and was observed to develop a significant plasma exposure during single and repeated-dose safety studies. Samples were analysed using a validated liquid chromatography-mass spectrometry (LC-MS/MS) assay. The peak concentration (C_{max}) and time to peak (T_{max}) were determined through visual inspection, and the linear trapezoidal rule was applied to calculate $AUC_{0\text{-}24h}$.

Primary outcome measure

The primary endpoint of this study is the difference between drugs (R317573 and lorazepam) and the difference between drugs and placebo on subjective measures relating to symptoms of anxiety using the VAS at peak effect of gas. Secondary endpoints for this study include the differences between drugs and the differences between drugs and placebo on all other subjective measures and cardiovascular function.

Statistics

A comparison of subjective measures of peak gas effects change from baseline was made using a Mann–Whitney *U* test for independent groups. For the 20-min gas inhalations, the mean value for systolic, diastolic blood pressure and heart rate were calculated and comparisons within drug treatments but between gases were made using paired *t*-test.

Results

Participants

In total, 90 healthy subjects were screened for potential inclusion. Of these 54 (60%) failed the screening procedure, and of these 29 (32% of total screened) failed as a result of not meeting the criteria of 'response' to 20 min of inhaled 7.5% CO_2, i.e. scoring $\geq$20 on the PSI. Of the 36 participants deemed suitable for inclusion, four did not return or decided not to participate due to changes in circumstances.

From the remaining subjects, 32 healthy participants were suitable for inclusion and were randomized into the study. Mean age was 25.7 years (SD: 4.06). Of these, 13 were male and 19 female. All were white, with a mean weight of 65.5 kg (SD: 10.8), and mean BMI of 21.8 (SD: 2.13). Participant completion and withdrawal information is presented in Table 2.

Subjective effects

The primary outcome measure is the subjective measures relating to symptoms of anxiety as measured by VAS.

Participants in both drug groups reported less CO_2-induced subjective symptoms compared with the

Table 2. Number and sex of participants who completed or withdrew from the study in each treatment group

Completed/Withdrawn	Placebo ($n=12$)	R317573 40 mg ($n=12$)	Lorazepam 2 mg ($n=8$)
Completed	11	12	8
M/F	6/5	3/9	3/5
Withdrawn (subject choice)	1	0	0
M/F	M		

Table 3. Mean and standard error for VAS measured at peak response of gas. Values are change from -15 baseline value for both gases, so negative values = decrease from baseline

					Mann–Whitney *U*-test	
VAS	GAS	PLAC ($n=11$)	R317573 40 mg ($n=12$)	LZP 2 mg ($n=8$)	*Z*	*p*-value
Fear	Air	8.2 (5.1)	−4.2 (1.6)*p	−1.2 (1.2)	−2.5	<0.05
	CO_2	14.1 (6.3)	3.3 (3.9)	2.5 (3.7)		
Relaxed	Air	−16.8 (6.3)	−2.1 (6.8)*l	3.1 (4.1)	−2.6	<0.01
	CO_2	−33.6 (6.7)	−29.2 (7.5)*l	−1.9 (6.1)*p	*−2.4	<0.05
					**−2.9	<0.01
Anxious	Air	5.4 (6.5)	0.4 (3.7)	−0.6 (2.4)		
	CO_2	17.3 (6.2)	10.0 (5.7)	8.7 (7.2)		
Happy	Air	−15.9 (4.6)	−10.0 (4.1)	0 (8.7)	*p−2.2	<0.05
	CO_2	−25.0 (7.3)	−24.2 (5.9)*l	−2.5 (7.0)*p	*1−2.0	0.05
Feel like leaving	Air	9.1 (4.9)	−7.9 (6.5)	−1.2 (2.9)		
	CO_2	12.7 (5.1)	7.1 (7.4)	3.1 (3.9)		
Stressed	Air	−0.7 (5.8)	−5.6 (4.8)	−3.3 (5.6)		
	CO_2	7.9 (10.4)	10.0 (9.6)	5.0 (9.2)		
Tense	Air	4.5 (4.8)	−1.7 (4.1)	−1.2 (2.3)	−1.9	0.062
	CO_2	15.9 (6.6)	10.8 (8.0)+l	0 (5.3)		
Nervous	Air	5.0 (5.5)	−5.4 (2.8)	1.2 (2.3)		
	CO_2	11.4 (6.0)	−1.7 (4.2)	1.9 (2.3)		
Irritable	Air	2.3 (3.8)	0 (3.8)	0 (3.3)		
	CO_2	5.9 (5.8)	8.7 (8.5)	5.0 (3.3)		
Worried	Air	3.2 (4.4)	−2.9 (4.0)	−4.4 (2.6)		
	CO_2	12.7 (6.9)	2.9 (5.6)	1.9 (4.6)		

* = significantly different, + = trend only; p = compared with placebo, l = compared with lorazepam. LZP 2 mg = lorazepam 2 mg, PLAC = placebo.

placebo group. These differences reached statistical significance for differences in VAS measures of fear, feel like leaving the room, tense and relaxed and happy (see Table 3 and Figure 1). The PSI and GAD-C questionnaires also showed significant between group differences (see Table 4). The SSAI was used to assess anticipatory anxiety prior to each gas inhalation, and there were no significant differences between gases or drug conditions.

Safety data

Treatments were well tolerated, and there were no clear, consistent treatment- or time-related changes in mean laboratory parameters (biochemistry, haematology, urinalysis) and vital signs. All ECGs were considered clinically normal. One participant from the placebo group withdrew on day 3 of the study due to treatment-emergent side effects (Table 5).

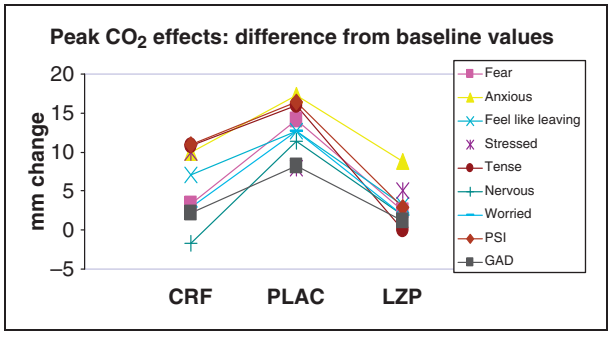

Figure 1. Shows that for all subjective variables measured, both the corticotrophin-releasing factor (CRF) antagonist and lorazepam (LZP) reduced CO_2-induced anxiety-related symptoms compared with placebo (PLAC).

Table 4. Mean and standard error for PSI and GAD-C questionnaire measured at peak response of gas. Values are change from -15 baseline value

					Mann–Whitney *U*-test	
Questionnaire	GAS	PLAC ($n=11$)	R317573 40 mg ($n=12$)	LZP 2 mg ($n=8$)	Z	*p*-value
PSI	Air	11.4 (4.4)	3.4 (1.2) + l	1.0 (1.7)	−1.9	0.062
	CO_2	16.4 (3.1)	11.0 (2.6)*l	2.9 (3.0)	−2.4	<0.05
GAD-C	Air	5.3 (2.1)	0.3 (0.6)	0.6 (0.1)	–	
	CO_2	8.2 (2.2)	2.2 (1.5)*p,l	1.1 (1.5)	p−2.2	0.05
					l−2.0	0.05

* = significantly different, + = trend only; p = compared with placebo, l = compared with lorazepam. LZP 2 mg = lorazepam 2 mg, PLAC = placebo.

Table 5. Number and percentage of treatment-emergent adverse events

	Placebo ($N=12$) *n*(%)	R317573 40 mg ($N=12$) *n*(%)	Lorazepam 2 mg ($N=8$) *n*(%)	Total ($N=32$) *n*(%)
Total no. subjects with adverse events	10 (83.3)	12 (100)	6 (75.0)	28 (87.5)
Most commonly occurring (>10% of population):				
Headache	4 (33.3)	3 (25.0)	3 (37.5)	10 (31.3)
Nausea	2 (16.7)	2 (16.7)	1 (12.5)	5 (15.6)
Somnolence	3 (25.0)	0	1 (12.5)	4 (12.5)
Dizziness	1 (8.3)	0	2 (25.0)	3 (9.4)
Dysmenorrhoea	0	3 (25.0)	0	3 (9.4)

Table 6. Mean and standard error () values for 20 minutes of gas inhalation

Measure	GAS	PLAC ($n=10$)	R317573 40 mg ($n=12$)	LZP 2 mg ($n=8$)
SBP	Air	136.5 (14.6)	127.5 (14.8)	129 (20.5)
	CO_2	150.8 (18.3)	144.4 (22.4)	140 (22.5)
		$p<0.05$	$p<0.01$	$p<0.01$
DBP	Air	77.9 (14.2)	70 (8.4)	74.5 (13.3)
	CO_2	86.2 (13.6)	77 (9.9)	83.4 (13.4)
		$p<0.05$	$p<0.01$	$p<0.05$
HR	Air	74.8 (13.3)	72.7 (7.9)	72.1 (10.9)
	CO_2	78.2 (13.5)	76.1 (6.6)	74.9 (8.7)
		ns	$p<0.05$	ns

ns = not significant. *p* = level of significance in paired samples *t*-test. SBP, systolic blood pressure, DBP, diastolic blood pressure; HR, heart rate.

Physiological data

Analysis of mean systolic and diastolic blood pressure during the 20-min air and 7.5% CO_2 inhalation revealed a significant difference between the gases for all drug groups. The 7.5% CO_2 increased heart rate when compared with air on the CRF group only (Table 6).

Pharmacokinetics

Following dosing of R31753 40 mg daily for 7 days, values (ng/ml) of plasma concentrations of R317573 and its acid metabolite R337676 are shown in Table 7.

Discussion

These results show the first demonstration of the effects of a CRF_1 receptor antagonist to reduce anxiety-related subjective symptoms in a human experimental model of anxiety. The drug was safe and well tolerated with few adverse events. There were differences between drug treatments and between drugs and placebo, but due to the sample sizes and variability within groups only a few of these differences reach statistical significance. However, since the study protocol was powered to continue if there was insufficient signal, when the number of participants or dose of R317573 was to be increased, the

Table 7. Mean plasma concentrations of R317573 and R337676

			R317573		R337676	
Day	Time	*N*	Mean (ng/ml)	SD	Mean (ng/ml)	SD
7	0 h	12	9.3	2.8	1,533	695
	1 h	11	34.1	19.8	1,578	743
	1.5 h	12	39.5	12.7	1,650	615
	2 h	12	45.2	18.9	1,755	708
8	Pre-CO_2	12	13.5	3.8	1,672	643
	0.5 h post	12	12.6	3.4	1,655	713
	1 h post	12	12.5	3.2	1,560	663

study sponsor made the decision to discontinue at this stage due to the positive data from this phase of the study.

The physiological changes, i.e. increases in blood pressure and heart rate in response to CO_2, were as expected and in line with our previous studies. R317573 did not appear to affect this response, although in this group heart rate was statistically significantly different after breathing CO_2 compared with air, although the values of change were very minimal and not clinically significant. Similarly, respiratory rate increased in line with the effects of CO_2 and were not influenced by treatment.

There was no evidence of any 7.5% CO_2 changes in stress hormones in this study, a finding which is consistent with similar observations in previous studies using the 7.5% CO_2 protocol (Bailey et al., 2007b). Moreover, the CRF antagonist had no impact on the basal levels of either ACTH or cortisol.

The dose of R317573 may be an issue with regard to the attenuation of subjective effects. We chose lorazepam 2 mg as an active control since we have repeatedly shown its efficacy in the 7.5% CO_2 model of anxiety in healthy participants, though with a considerable amount of sedation. It would have been surprising had R317573 had the same robust anxiolytic profile, given the sedating and muscle relaxant actions of lorazepam. However, perhaps an increased dose or a within-subject design would further strengthen this preliminary evidence of efficacy. Although, it should be noted that a recent study of the CRF_1 antagonist, pexacerfont, in female patients with GAD, failed to demonstrate efficacy compared with placebo (Coric et al., 2010).

This first proof-of-concept study using a putative anxiolytic in the CO_2 model of anxiety has shown some differences between treatments. However, there are limitations to these findings, particularly the use of separate groups unbalanced for number and sex. This tends to result in baseline differences within groups, making interpretation of the data less clear. Another potential flaw in these types of study examining fairly specific anxiety effects is the inclusion of intravenous cannulation and regular blood taking (which may result in blocked cannula or difficulty with extraction). This in itself is an anxiogenic stimulus for many, for if blood-letting is not forthcoming, the stress and discomfort can lead to adverse stress which leads to exclusion of outliers within the data set.

Another potential confounding factor was the requirement for all participants to sleep in the research unit overnight prior to the CO_2 study. It was decided that this would add an element of control to the study and ensure that all participants arrived for their test day on time, having eaten the same standardized breakfast. However, some participants reported that they did not sleep as well as they normally do, presumably due to the novel context of the hospital ward, which may have interfered with hormonal as well as subjective responses.

To conclude, using the 7.5% CO_2 model of anxiety, the anxiolytic efficacy of a novel CRF_1 receptor antagonist, R317573, has been indicated in this complex study without noticeable sedative actions.

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Conflict of interest

Schmidt and van der Ark were employees of J&J; Dourish and Dawson are employees of P1Vital Ltd; Nutt is a shareholder in P1Vital Ltd. The other authors have no conflict of interest.

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