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Evaluation of the effects of venlafaxine and pregabalin on the carbon dioxide inhalation models of Generalised Anxiety Disorder and panic

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Abstract

Previous studies have shown that subjective and objective symptoms of anxiety induced by 7.5% CO_2 inhalation can be attenuated by anxiolytics such as lorazepam and, to a lesser extent, paroxetine. Venlafaxine and pregabalin, two other licensed treatments for Generalised Anxiety Disorder, were used to further investigate the 7.5% and 35% CO_2 models of anxiety in healthy volunteers. Fifty-four participants were randomised to receive either placebo, venlafaxine or pregabalin. Study treatments were dosed incrementally over a three week period, to reach daily doses of 150mg venlafaxine and 200mg pregabalin by the CO_2 challenge test day. Participants inhaled air 7.5% CO_2 for 20 minutes (single-blind presentation), and a non-blinded single vital capacity of 35% CO_2. Subjective ratings were recorded before and after each inhalation. Both 7.5% and 35% CO_2 inhalations produced the expected effects of increased ratings of symptoms of panic and anxiety, with increased blood pressure and heart rate. No significant treatment effects were found, although there were trends towards a reduction in feeling tense and nervous by both drugs compared with placebo during the 7.5% CO_2 challenge, and a reduction in alertness generally in the venlafaxine group compared with the pregabalin group. In contrast with the clear anxiolytic effects of benzodiazepines reported in several previous CO_2 studies, these findings suggest that the anxiogenic effects of CO_2 challenges are not significantly influenced by these serotonergic and GABAergic anxiolytics. This may be due to a lack of sensitivity of the CO_2 challenges in healthy volunteers to these drug types.

Keywords

Anxiety, carbon dioxide, experimental human model, panic, pregabalin, venlafaxine

Introduction

Generalised Anxiety Disorder (GAD) is common, with a US survey using *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) criteria showing a lifetime prevalence as high as 6% (Kessler et al., 2005). A recent Chinese survey also using these criteria found a prevalence of 4.1%, with 72% of these patients also meeting criteria for major depression (Ying et al., 2010). Treatment can be complex, with many patients failing to respond to first-line treatments, requiring combination therapies or switching between treatments (Baldwin et al., 2005). Current treatments can have significant disadvantages, such as slow onset of action, abuse potential and unpleasant side effects (Davidson et al., 2010). There is a need for new evidence-based effective treatments, with a good side effect profile, to improve patient productivity and quality of life and reduce the burden on health care resources.

The CO_2 model of anxiety and panic may be useful in the development of such treatments. It has been shown to produce robust subjective and objective effects, some of which are similar to symptoms of GAD or panic, which may provide a useful tool to model anxiety in healthy volunteers for the purposes of anxiety research and new drug development (Bailey, 2003, 2005, 2007, 2009; Papadopoulos et al., 2010). These CO_2-induced effects can be attenuated by selective serotonin reuptake inhibitors (SSRIs), benzodiazepines and cognitive behaviourtherapy in panic disorder patients (e.g. Bertani, et al., 1997, 2001; Gorman et al., 1997; Nardi et al., 2000; Perna et al., 1997; Polset al., 1996; Sanderson et al., 1994; Schruers and Griez, 2004). Bailey and Nutt (2008) have postulated that a mechanism by which CO_2 inhalation can elicit anxiety responses is by reducing the amount of available gamma-amino butyric acid (GABA),both centrally and peripherally. It has been shown that GABA dysfunction may

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play a role in various anxiety disorders. $GABA_A$ receptors are targets for barbiturates, neurosteroids and alcohol, as well as benzodiazepines. In GAD, there is evidence of reduced benzodiazepine receptor function in the GABA system (Tiihonen et al., 1997), and drugs acting on the GABA system have been shown to be effective in treating GAD (Pohl et al., 2005).

The effect and efficacy of psychotropic medication has been examined by administering the 7.5% and 35% CO_2 challenges in healthy volunteers and patient groups with varying results. It has been hypothesised that different anxiolytic and antidepressant medication might have drug specific effects on CO_2 inhalations. In this study, we present data regarding the effects of two anxiolytics, to investigate the suitability of these CO_2 challenges as models of anxiety and panic, and perhaps in turn elucidate further the mechanisms of these CO_2 challenges. The first anxiolytic is pregabalin, which binds to the alpha-2-delta subunit of a voltage-gated calcium channel and inhibits neurotransmitter release (Gee et al., 1996), and the second is venlafaxine, a serotonin noradrenaline reuptake inhibitor (SNRI) that binds with high affinity to monoamine transporter sites and thereby increases the synaptic levels of serotonin and noradrenaline (and to a lesser extent dopamine) by preventing their reuptake (Holliday and Benfield, 1995).

Pregabalin is prescribed for epilepsy and neuropathic pain as well as GAD, and venlafaxine is licensed for depression and several anxiety disorders including GAD and panic disorder. Both venlafaxine and pregabalin have been shown to be effective treatments in GAD, and venlafaxine for panic disorder and social anxiety disorder (Bandelow et al., 2007; Kim et al., 2006; Liebowitz et al., 2005, 2009; Pohl et al., 2005; Pollack et al., 2007). A study by Montgomery et al. (2006) compared pregabalin and venlafaxine treatment for six weeks in a study of GAD patients and found similar efficacy for both drugs, with pregabalin achieving effectiveness after one week, a week before venlafaxine. Kelsey (2000) has also shown that venlafaxine requires administration for a couple of weeks before it is more effective than placebo, and Lydiard et al. (2010) found that reductions in HAM-A scores are observed following 300–600 mg/day pregabalin after one week of dosing. This suggests that venlafaxine and pregabalin, unlike benzodiazepine agonists such as lorazepam, are not immediately active and that dosing needs to be continuous for a period of time before they demonstrate clinical efficacy.

Methods

Ethical considerations

The study protocol was approved by the Cambridgeshire 2 Research Ethics Committee and local NHS Trusts, and performed in accordance with ICH Good Clinical Practice. All participants gave written informed consent after receiving a complete description of the study prior to their participation.

Power calculation

A power calculation of peak 7.5% CO_2 effects (absolute values) based on the mean Visual Analogue Scale ratings of anxiety of 31.2 (placebo) and 17.9 (lorazepam, 2mg), with a common standard deviation of 12 (Bailey et al., 2007), suggested that to detect a significant difference at the $p \leq 0.05$ level, 13 participants were required for this study. This number was increased to 18 in each arm to allow for variation in parameters between experiments and to balance the experimental design. As there were three arms, 54 participants were required in total.

Participants

Participants were recruited using existing databases at the Psychopharmacology Unit, University of Bristol, and via advertisements on the University careers website and on campus.

At screening, all participants passed strict inclusion and exclusion criteria before participation. Prior to inclusion, participants were given a physical examination by a study physician, including an electrocardiogram (ECG) and vital signs, and medical histories were taken. Participants were interviewed by a study physician to ensure no history of significant mental disorder or first degree relatives with severe anxiety or panic disorder. Other exclusion criteria were: current or history of drug or alcohol abuse or dependence, smoking more than five cigarettes per day, current or history of cardiovascular, respiratory or renal disease, hypertension, migraine or epilepsy, and participation in another CO_2 study within the last six months. An alcohol breath test was performed, and a urine sample was tested to screen for drugs of abuse, pregnancy in females and for urinalysis. A blood sample was taken to screen for general health problems. Medication was not allowed for two weeks prior to and during testing, unless deemed by the investigator not to interfere with the study or compromise safety. No alcohol or illicit drugs were allowed for the duration of the whole study. Participants' family doctors were informed of their participation and participants were compensated £400 for their time.

Study design and procedure

This was a randomised, double-blind, placebo-controlled, parallel design study. Baseline measures of trait anxiety and personality were taken using the Anxiety Sensitivity Index (ASI) (Reiss and McNally, 1985), Spielberger State Trait Inventory –Trait (STAI) (Spielberger, 1983), the Eysenck Personality Questionnaire – Revised (EPQ-R) (Eysenck et al., 1985) and Cloninger's Temperament and Character Inventory-125 (TCI) (Cloninger et al., 1994). Participants were randomised to receive venlafaxine, pregabalin or placebo over a 26 day period.

After randomisation, participants were asked to keep a diary of adverse events and dose times, and attended the research facility (the Psychopharmacology Unit clinical testing rooms at the Bristol Royal Infirmary) weekly for a brief health and compliance check (breath alcohol, urine drugs of abuse screen, temperature and blood pressure). After three weeks of dose-increasing treatment, participants underwent the CO_2 challenge procedure. This consisted of the inhalation of air for 20 minutes, 10 minutes' rest, the inhalation of 7.5% CO_2 for 20 minutes, 10 minutes' rest, inhalation of 35% CO_2 for one breath (vital capacity inhalation) and 30 minutes' rest. During the challenge, subjective and physiological measures were collected. For five days after the CO_2 test day, dose levels were decreased and participants attended a follow-up health check once withdrawn from the drug.

Study drugs

The study treatments were venlafaxine, pregabalin and placebo. The active treatments were over-encapsulated to give the appearance of the red placebo capsule. Treatments were dose-increased

to avoid adverse events over a three week period, to reach doses of 150mg venlafaxine and 200mg pregabalin by day 21 (CO_2 test day). These doses were chosen to be within therapeutic range, but it was considered more important that the drugs had minimal side effects in this healthy volunteer sample, rather than being exactly equipotent. Studies of GAD patients by Davidson et al. (1999) and Pohl et al. (2005) have shown that these doses reduce HAM-A scores compared with placebo. British National Formulary recommended doses for anxiety range between 75mg and 225mg of venlafaxine and 150mg and 600mg daily of pregabalin.

The dose of venlafaxine was 75mg on days 0–2, 112.5mg on days 3–6, 150mg on days 7–21, 75mg on days 22–24 and 37.5mg on days 25–26. The dose of pregabalin was 100mg on days 0–6, 200mg on days 7–21, 100mg on days 22–24 and 50mg on days 25–26. The last administration of the maximum dose of each drug was on the day of the CO_2 challenge. Doses were taken as two capsules daily. The study drugs were provided in blister packs and participants were instructed to take one capsule every morning and one capsule every evening with food. Capsules were taken in the order set out on the blister pack (day 1 morning, day 1 evening, day 2 morning, day 2 evening, etc.). The drugs were labelled according to the point in the study when they were taken.

The first doses of study medication, and the doses on the CO_2 test day (day 21), were administered on-site under direct supervision of two researchers. No other doses were witnessed, as participants were given the study medication to take home and self-administer, noting the times of doses and any side effects in a diary. Participants were required to bring their diaries and full or empty drug packaging with them at each visit for a compliance check that all medication had been taken, or to note when participants had forgotten to take their medication. If participants forgot to take more than two capsules in one week, they would have been withdrawn from the study for non-compliance.

Delivery of gas

The delivery of gas followed procedures previously reported by Bailey et al. (2009) and Papadopoulos et al. (2010). Participants were seated comfortably during inhalations and at least two investigators attended the sessions, with one investigator remaining in sight of the participant throughout the procedure.

For the 20 minute inhalations, the gas mixtures used were an inhalation of CO_2 7.5%/O_2 21%/N 71.5% and an inhalation of medical air, delivered via a nasal–oral exercise facemask (Hans Rudolf, Kansas) attached to a 500 L Douglas bag via tubing. Gas flow was monitored to allow for a reservoir of gas in the bag at all times. Gas cylinders were kept out of view in a separate room to blind the participant as to the order of gas presentation, which was single blind. Air was always presented first but participants were informed that presentation was random to avoid expectation effects and differing levels of anticipatory anxiety.

The gas mixture used for the vital capacity single breath inhalation was CO_2 35%/O_2 65%. Full instructions were given before participants underwent this non-blinded inhalation. First, participants were asked to insert a mouthpiece attached to a small Douglas bag. A nose clip was used to ensure participants only breathed the contents of the bag. After expiring fully, a full vital capacity inhalation was taken and held for a count of four seconds. The equipment was then removed and participants were asked to breathe normally.

Subjective measures

Participants scored Visual Analogue Scales (VASs) verbally on a scale of 0 (not at all) to 100 (the most ever) using the adjectives: alert, fearful, relaxed, anxious, happy, feel like leaving the room, stressed, tense, nervous, irritable and worried.

The Panic Symptom Inventory (PSI) was used to rate panic anxiety and the associated symptoms of autonomic arousal, with the option of rating 0 = not at all, 1 = slight, 2 = moderate, 3= severe, 4 = very severe. The PSI was adapted from Clark and Hemsley (1982) and lists 34 items. It has been used in studies of panic provocation (Bell et al., 2002; Nutt et al., 1990) and previous CO_2 studies (Argyropoulos et al., 2002; Bailey et al., 2005, 2007). The Generalised Anxiety Disorder Criteria Inventory (GAD-C) was used to measure participants' current anxiety state.

PSI, GAD-C and VAS ratings were performed 30 minutes (baseline) and 15 minutes before inhalations and at the end of the session (30 minutes after the last (35% CO_2) inhalation). Immediately after each inhalation, participants were asked to rate how they felt when the effects of the gas were at their greatest, this rating being 'peak'.

The Spielberger State Trait Anxiety Inventory – State (SSAI) (Spielberger, 1983) was used to measure state anxiety and was administered 10 minutes prior to each inhalation and at the end of the testing session.

Objective measures

Continuous measurements of blood pressure and heart rate were obtained during all inhalations using the Finapres (Ohmeda, Englewood, CO). For full details see Coupland et al. (1995), but briefly, the participants wore a finger cuff with a photosensitive cell connected via a servo-controlled pump, which inflated the cuff to maintain a constant pressure on the finger. To avoid artefact, the participant was instructed to stay still and not to move their hand or cross their legs. Data were captured onto a DOS-based program and later analysed using software designed for the purpose.

Statistical analyses

Comparisons of differences between baseline (at the beginning of the test session before sight of the inhalation equipment) and peak gas effects (peak air minus baseline versus peak 7.5% CO_2 minus baseline versus peak 35% CO_2 minus baseline) were made for subjective variables. Also, direct comparisons between peak 7.5% and peak 35% CO_2 subjective ratings were made. Blood pressure and heart rate were averaged over the air and 7.5% CO_2 inhalations and compared. No baseline cardiovascular measures were taken using the Finapres. For the 35% CO_2 inhalation, blood pressure and heart rate 30 seconds before inhalation, at the point of inhalation and 30 seconds after inhalation were compared. Physiological measures during the 35% CO_2 inhalation for two participants contained too many artefacts to be included in the analysis, so here 17 participants were included in the analysis for the venlafaxine and pregabalin groups. Subjective and objective variables were analysed using mixed model and repeated measures ANOVAs respectively for Drug, Gas and Drug*Gas interactions using the Greenhouse-Geisser correction. Although not all VAS variables were normally distributed, it is assumed that the test is robust enough to withstand such violations. Post hoc

Table 1. Participant demographics and characteristics at baseline.

	Measure	Placebo	Venlafaxine	Pregabalin	Matched population norm
	Age	22.0 (2.47)	22.8 (2.85)	24.4 (7.11)	
	Gender	9 M, 9 F	12 M, 6 F	8 M, 10 F	
	ASI	14.7 (7.27)	15.7 (7.79)	12.2 (8.45)	19.1 (9.1)
	STAI	35.3 (9.51)	35.2 (6.40)	33.2 (7.20)	35.9 (9.7)
EPQ-R	Psychoticism	5.5 (2.81)	8.1 (3.48)	6.3 (3.04)	7.4 (4.2)
	Extraversion	16.3 (3.08)	16.8 (3.93)	16.4 (4.45)	14.3 (5.2)
	Neuroticism	6.2 (4.19)	5.4 (3.65)	6.8 (4.68)	11.8 (5.1)
	Lie	7.3 (3.01)	7.1 (3.46)	6.1 (3.08)	5.9 (3.6)
	Addiction	7.4 (3.53)	7.8 (2.53)	8.2 (3.29)	12.1 (4.6)
	Criminality	7.2 (3.23)	7.6 (3.45)	7.6 (3.81)	9.0 (4.5)
TCI-125	Novelty seeking	10.3 (3.10)	11.7 (4.46)	10.3 (4.07)	9.8 (4.1)
	Harm avoidance	6.9 (4.56)	5.6 (3.62)	5.7 (4.65)	9.3 (5.2)
	Reward dependence	11.3 (2.52)	9.8 (3.09)	10.3 (3.41)	10.8 (2.8)
	Persistence	1.9 (1.73)	2.2 (1.73)	2.6 (1.75)	3.0 (1.7)
	Self-directedness	20.4 (5.07)	18.1 (2.86)	20.3 (3.40)	16.9 (5.3)
	Cooperativeness	21.7 (2.85)	22.4 (2.68)	21.9 (3.08)	21.0 (3.8)
	Self-transcendence	3.7 (2.40)	6.3 (3.74)	3.9 (4.25)	6.5 (3.8)

ASI: Anxiety Sensitivity Index; STAI: Spielberger State–Trait Anxiety Inventory; EPQ-R: Eysenck Personality Questionnaire – Revised; TCI-125: Cloninger's Temperament and Character Inventory (125 items). Normal values taken from Peterson and Reiss (1992; ASI); Spielberger (1983; STAI); Eysenck and Eysenck (1991; EPQ-R) and Cloninger et al. (1991; TCI-125). Table shows mean scores with standard deviations in parentheses.

analyses were simple main effects and pairwise comparisons with Bonferroni correction unless otherwise stated. All data were analysed using SPSS Version 16.0 for Windows.

Results

Participants

We were contacted by 282 volunteers, of which 115 underwent an initial brief telephone questionnaire. Seventy-nine of these volunteers were screened, and 60 were suitable for study inclusion and randomised. Six participants withdrew from the study because of adverse events; five were in the venlafaxine group and one was in the placebo group. These participants were replaced to make a total of 54 completing participants, 29 male and 25 female, aged between 20 and 43 years of age (mean age 23.1, standard deviation (SD) 4.68). Each treatment group consisted of 18 participants.

Chi-square tests showed that there was no significant difference between numbers of males and females in each treatment group, and no significant difference between numbers of each ethnic origin in each treatment group. One way ANOVAs showed there was no significant difference between the treatment groups with respect to age, usual caffeine and alcohol intake, usual amounts of smoking, body mass index and scores on the ASI (Reiss and McNally, 1985), STAI (Spielberger, 1983) and all subscales of the EPQ-R (Eysenck et al., 1985) and TCI-125 (Cloninger et al., 1994, see Table 1). There was also no significant difference between compliance (the numbers of capsules missed) by participants in each treatment group (an average throughout the study of 0.9 capsules in the placebo group, 0.8 in the venlafaxine group and 0.7 in the pregabalin group). Capsules were missed because participants forgot to take them.

Questionnaires

A two-way mixed model ANOVA of the SSAI showed anxiety levels returned to baseline (pre-air) before each inhalation and at the end of the test session, with the exception of prior to the 35% CO_2 inhalation, where anxiety levels were significantly higher than at all other time points ($F(3,54)= 22.19$, $p<0.001$; Bonferroni pairwise comparisons: pre-air versus pre-7.5% CO_2, $p=0.135$; pre-7.5% versus pre-35% CO_2, $p<0.001$; pre-air versus pre-35% CO_2, $p<0.001$). This is likely to be anticipatory anxiety as the participants knew they were about to receive an inhalation of a high CO_2 concentration. There was no significant difference in SSAI between drug groups at this time point. Scores on average were 31 at baseline (SD 6.9), 32 before 7.5% CO_2 (SD 7.1), 36 before 35% CO_2 (SD 9.1) and 31 at the end of the session (SD 8.3). For comparison, average state anxiety score for males and females aged 19 to 39 years is 36 (SD 10.6) (Spielberger, 1983).

Accounting for baseline and compared with air, the inhalation of 7.5% CO_2 significantly increased the total PSI scores ($F(1,51)=62.4$, $p<0.001$). Compared with baseline, the inhalation of 35% CO_2 also significantly increased the total PSI scores ($F(1,51)=149.5$, $p<0.001$). There was a trend towards a Drug*Gas interaction ($F(2,51)=2.4$, $p=0.097$), whereby scores in the pregabalin group were lower than the venlafaxine group at peak 35% CO_2 effects. Accounting for baseline and compared with air, 7.5% CO_2 inhalation significantly increased GAD Criteria Inventory (GAD-C) scores ($F(1,51)=40.7$, $p<0.001$), and compared with baseline, inhalation of 35% CO_2 also significantly increased GAD-C scores ($F(1,51)=105.6$, $p<0.001$), but there was no significant difference between scores for the two CO_2 inhalations overall. However, by treatment group, GAD-C scores on venlafaxine were significantly higher at peak 35% compared with peak 7.5% ($t(17)=2.14$, $p<0.05$). There were no significant differences between treatment (see Figures 1 and 2).

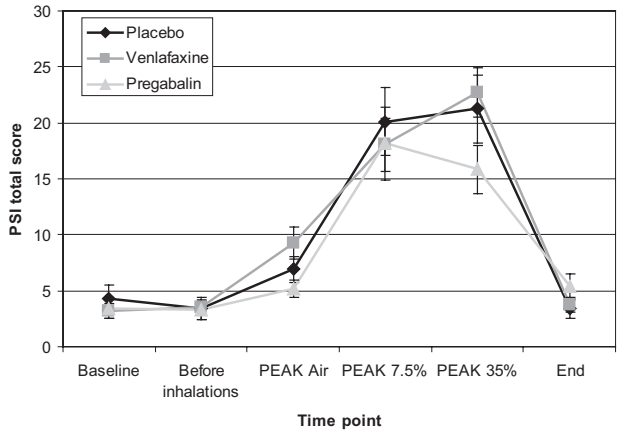

Figure 1. Graph shows the total Panic Symptom Inventory (PSI) score over time in the three drug conditions.

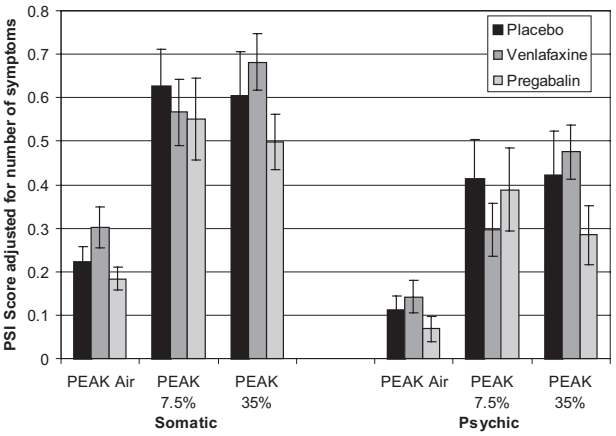

Figure 3. Graph shows the scores for Panic Symptom Inventory (PSI) Somatic and Psychic symptoms at the peak effects of the three inhalations.

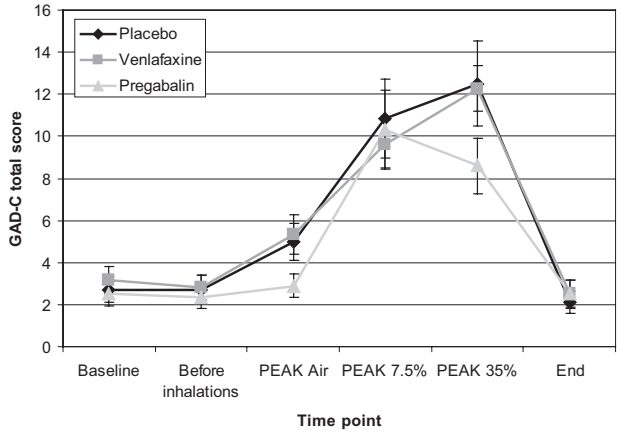

Figure 2. Graph shows the total Generalised Anxiety Disorder Criteria Inventory (GAD-C) scores over time in the three drug conditions.

Dividing the PSI into somatic (26 symptoms, e.g. sweating, heart racing) and psychic (nine symptoms, e.g. going mad, apprehension) symptoms, and taking account of the difference in the number of symptoms queried, paired *t*-tests showed no significant differences between the CO_2 inhalations and air on venlafaxine or pregabalin. However, on placebo, there was a non-significant trend towards more somatic reports of anxiety than psychic during the 7.5% CO_2 inhalation ($t(17)=1.786$, $p=0.092$), but not the 35% CO_2 inhalation (see Figure 3).

Visual Analogue Scales

The 7.5% CO_2 inhalation (accounting for baseline scores and compared with air inhalation) and the 35% CO_2 inhalation (compared with baseline) significantly increased ratings at peak of feeling fearful, anxious, like leaving the room, stressed, tense, nervous, irritable and worried, and decreased ratings of feeling relaxed and happy (see Table 2).

There were no significant main effects of drug for any VAS measure, probably because the variances were large. However, there were significant Gas*Drug interactions for the ratings of feeling tense ($F(2,51)=3.9$, $p<0.05$) and nervous ($F(2,51)=3.4$, $p<0.05$) peak 7.5% CO_2 compared with peak air (accounting for baseline). One way ANOVAs showed trends towards feeling less tense during this inhalation ($F(2,51)=2.9$, $p=0.062$) on pregabalin compared to placebo (Tukey's, $p=0.065$), and less nervous ($F(2,51)=2.8$, $p=0.072$) on pregabalin and venlafaxine compared with placebo (Tukey's post hoc analysis was not significant; $p=0.120$ and $p=0.108$ respectively). Taking account of baseline, direct comparison of the 7.5% CO_2 and 35% CO_2 inhalations by paired *t*-tests revealed that participants felt more like leaving the room ($t(53)=3.13$, $p<0.01$) and more irritable ($t(53)=2.67$, $p<0.05$) during the 7.5% CO_2 inhalation compared with the 35% CO_2 inhalation, indicating it was a less pleasant experience. All means and standard deviations are shown in Table 3.

Alertness ($F(2,51)=4.1$, $p<0.05$) was increased, compared with air, during the 7.5% CO_2 inhalation but not the 35% CO_2 inhalation. During the 7.5% CO_2 inhalation, alertness was significantly reduced by venlafaxine compared with pregabalin (pairwise comparisons, $p<0.05$) and there was a trend towards reduced alertness by venlafaxine compared to placebo (pairwise comparisons, $p=0.076$). There was a trend towards reduced alertness generally ($F(2,54)=2.86$, $p=0.067$) in the venlafaxine group compared with the pregabalin group ($p=0.094$) (see Figures 4 and 5).

Physiological

Physiological measures taken during weekly health checks and at the beginning of the CO_2 challenge day showed blood pressure and heart rate were significantly higher in the venlafaxine group compared with pregabalin and placebo (data not shown). Compared with air, the 7.5% CO_2 inhalation significantly increased systolic blood pressure (SBP) ($F(1,54)=67.89$, $p<0.001$), diastolic blood pressure (DBP) ($F(1,54)=40.70$, $p<0.001$) and heart rate (HR) ($F(1,54)=30.73$, $p<0.001$). Significant effects of drug were also found for SBP ($F(2,54)=13.82$, $p<0.001$), DBP ($F(2,54)=11.43$, $p<0.001$) and HR ($F(2,54)=6.07$, $p<0.01$) in that SBP, DBP and HR were significantly higher in the venlafaxine group compared with the pregabalin and placebo groups (all *p* values <0.05). There were no Drug*Gas interactions.

Table 2. Effect of CO2 inhalations on Visual Analogue Scale (VAS) ratings.

	Peak 7.5% CO_2 - baseline vs. peak air - baseline			Peak 35% CO_2 - baseline vs. peak air - baseline		
VAS variable	*F*	*p* value	Effect	*F*	*p* value	Effect
Alert	$F(1,51)=4.1$	$p<0.05$	↑	$F(1,51)=0.7$	$p=0.508$	NS
Fearful	$F(1,51)=32.7$	$p<0.001$	↑	$F(1,51)=37.7$	$p<0.001$	↑
Relaxed	$F(1,51)=63.6$	$p<0.001$	↓	$F(1,51)=102.1$	$p<0.001$	↓
Anxious	$F(1,51)=40.4$	$p<0.001$	↑	$F(1,51)=23.3$	$p<0.001$	↑
Happy	$F(1,51)=45.2$	$p<0.001$	↓	$F(1,51)=70.5$	$p<0.001$	↓
Leaving	$F(1,51)=31.4$	$p<0.001$	↑	$F(1,51)=10.3$	$p<0.01$	↑
Stressed	$F(1,51)=37.7$	$p<0.001$	↑	$F(1,51)=27.8$	$p<0.001$	↑
Tense	$F(1,51)=45.7$	$p<0.001$	↑	$F(1,51)=42.1$	$p<0.001$	↑
Nervous	$F(1,51)=31.8$	$p<0.001$	↑	$F(1,51)=31.7$	$p<0.001$	↑
Irritable	$F(1,51)=14.5$	$p<0.001$	↑	$F(1,51)=8.0$	$p<0.01$	↑
Worried	$F(1,51)=21.6$	$p<0.001$	↑	$F(1,51)=30.4$	$p<0.001$	↑

Table 3. Table showing the effect of CO_2 inhalations on Visual Analogue Scale (VAS) ratings by drug treatment.

VAS variable	Peak air - baseline			Peak 7.5% CO_2 - baseline			Peak 35% CO_2 - baseline		
	Placebo	Venlafaxine	Pregabalin	Placebo	Venlafaxine	Pregabalin	Placebo	Venlafaxine	Pregabalin
Alert	−18.3 (15.72)	−27.2 (19.87)	−9.4 (17.14)	0.0 (22.82)	−19.4 (25.37)	−4.7* (27.36)	−7.8 (24.98)	−17.2 (35.74)	−6.1 (28.05)
Fearful	0.0 (11.38)	0.8 (8.09)	1.9 (9.26)	17.8 (22.57)	13.3 (17.24)	14.7 (18.75)	15.6 (25.14)	25.6 (20.93)	14.4 (20.07)
Relaxed	−0.8 (23.47)	−2.5 (22.51)	−10.8 (21.44)	−37.8 (21.71)	−27.5 (24.51)	−32.8 (28.66)	−39.2 (21.64)	−41.1 (30.71)	−36.7 (31.62)
Anxious	−1.9 (11.65)	−3.3 (18.15)	0.8 (11.79)	22.8 (24.57)	9.7 (23.54)	16.1 (22.66)	15.0 (20.22)	13.1 (27.34)	15.3 (17.19)
Happy	−12.2 (14.47)	−13.3 (13.50)	−9.4 (7.84)	−32.8 (22.83)	−25.3 (15.48)	−30.0 (21.49)	−27.2 (23.21)	−33.9 (30.08)	−28.6 (24.72)
Leaving	0.6 (8.02)	9.7 (12.30)	−0.3 (14.09)	18.6 (23.31)	29.2 (30.01)	21.9 (27.71)	3.3 (15.43)	13.9 (26.71)	14.2 (27.77)
Stressed	−3.9 (10.79)	1.9 (9.87)	−0.3 (11.56)	18.9 (25.64)	17.8 (22.31)	14.7 (22.85)	12.8 (26.58)	16.9 (17.33)	18.9 (22.85)
Tense	1.1 (11.70)	7.5 (12.63)	−0.8 (7.91)	30.3 (22.52)	18.3 (15.81)	15.0 (20.79)	21.1 (26.98)	24.2 (20.60)	18.3 (24.07)
Nervous	−0.8 (11.28)	0.3 (11.31)	1.4 (9.20)	21.4 (22.87)	9.7 (10.91)	10.0 (14.95)	19.7 (26.92)	18.3 (25.15)	20.6 (24.37)
Irritable	4.2 (8.45)	5.3 (14.40)	−0.3 (11.69)	15.0 (19.10)	12.2 (21.09)	13.6 (23.57)	6.9 (18.95)	6.7 (18.71)	6.1 (13.01)
Worried	−0.6 (11.62)	0.3 (10.50)	−1.9 (11.00)	15.8 (22.83)	7.2 (12.63)	9.2 (16.38)	14.2 (25.16)	19.7 (19.74)	15.6 (20.64)

Table shows mean scores of the differences between inhalation and baseline with standard deviations in parentheses.
*Pregabalin significantly different from venlafaxine ($p<0.05$).

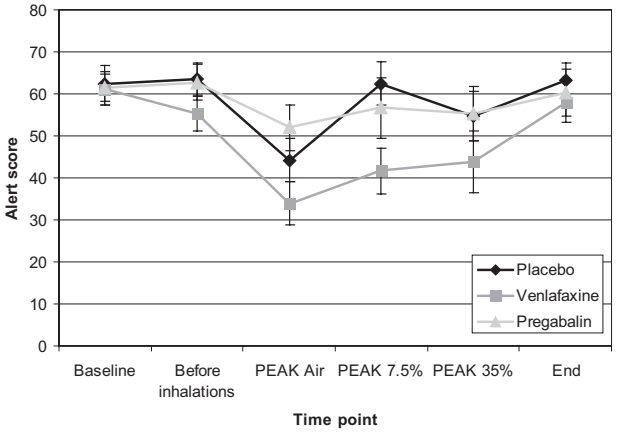

Figure 4. Graph shows ratings of Visual Analogue Scales (VAS) Alertness over time in the three drug conditions.

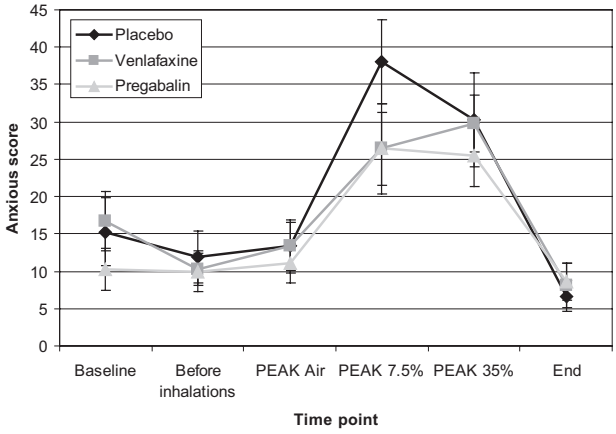

Figure 5. Graph shows ratings of VAS Anxious over time in the three drug conditions. Although non-significant, this pattern is also seen in all negative effect VASs, the reverse seen in positive effect VASs.

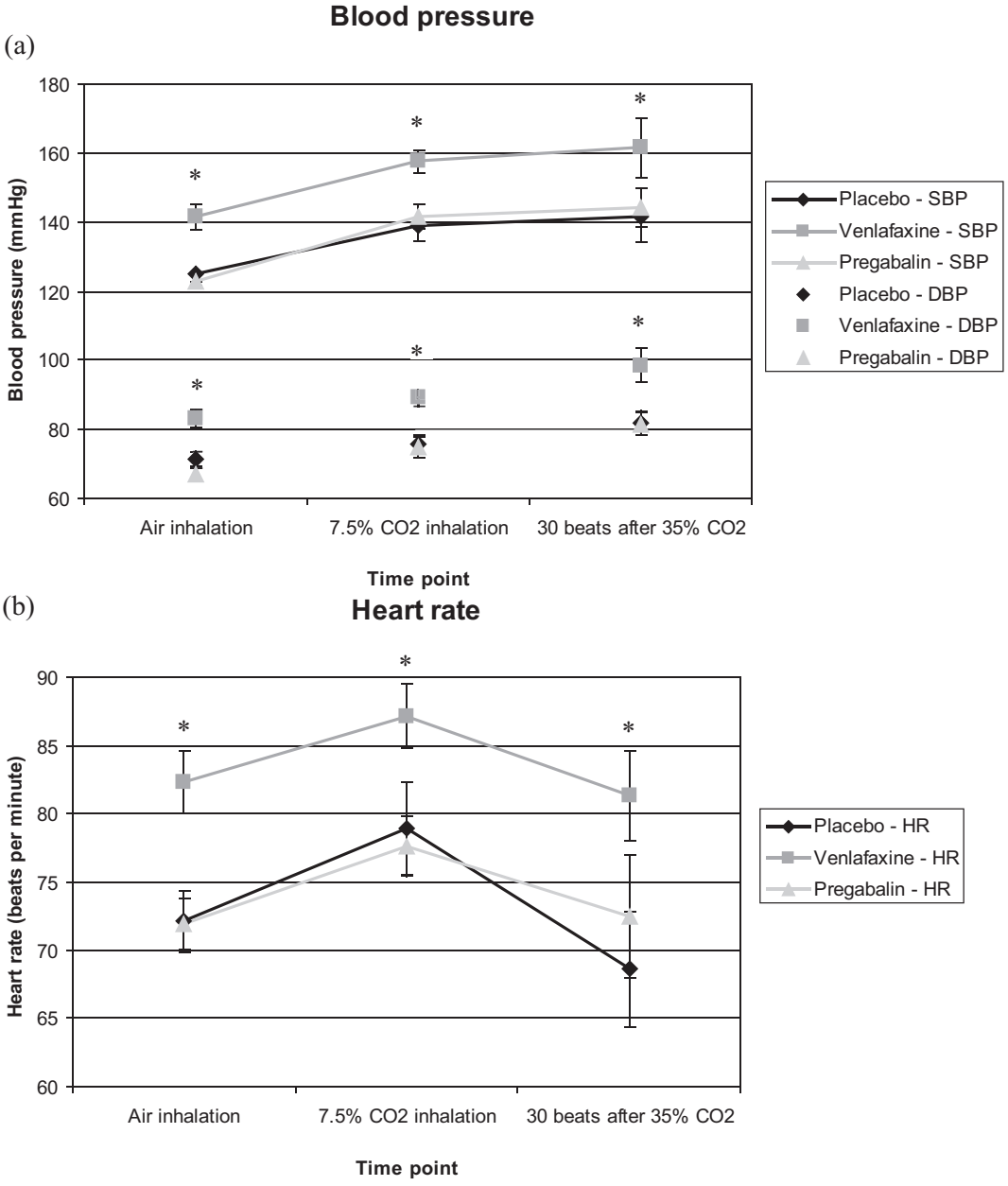

Figure 6. Graph shows that systolic (SBP) and diastolic (DBP) blood pressure (a) and heart rate (HR) (b) were significantly elevated in the venlafaxine group compared with the pregabalin and placebo groups.
*$p<0.05$

Analysis of the 35% CO_2 inhalation showed significant Time ($F(2,52)=13.13$, $p<0.001$) and Drug ($F(2,52)=3.47$, $p<0.05$) effects of SBP, and Time ($F(2,52)=9.60$, $p<0.001$) and Drug ($F(2,52)=8.85$, $p<0.01$) effects of HR. SBP and HR were significantly higher after inhalation compared with before inhalation and the point of inhalation (p values <0.01) and significantly higher on venlafaxine compared with pregabalin (p values <0.05) and also placebo for HR (p values <0.05).

There were also significant Time ($F(2,52)=21.48$, $p<0.001$) and Drug ($F(2,52)=3.32$, $p<0.05$) effects of DBP, in that DBP was significantly higher after 35% CO_2 inhalation compared with before inhalation and the point of inhalation, and DBP at the point of inhalation was significantly higher than before inhalation (p values <0.05). DBP was significantly higher on venlafaxine compared with pregabalin ($p<0.05$). There was a significant Time*Drug interaction ($F(4,52)=3.93$, $p<0.01$). A one-way ANOVA showed a significant difference in DBP between the drug groups 30 seconds after inhalation ($F(2,52)=5.81$, $p<0.01$). Tukey HSD analysis showed DBP for the venlafaxine group was significantly higher at this time point than for the pregabalin and placebo groups (p values <0.05). This indicates that the increase in DBP associated with the 35% CO_2 inhalation was significantly greater in the venlafaxine group. See Figure 6(a) and (b).

Adverse events (AEs) and concomitant medications

In total, 317 adverse events (AEs) were reported after randomisation by 48 of the 54 participants. Six participants reported no AEs at any point during the study. Four of these participants were taking pregabalin, one was taking placebo and one was taking venlafaxine.

There were 79 AEs reported in the placebo group (86% mild and 14% moderate, as judged by the volunteer according to predefined criteria), 143 reported in the venlafaxine group (81% mild and 18% moderate) and 95 reported in the pregabalin group (89% mild and 10% moderate). Pregabalin was better tolerated than venlafaxine with respect to number and severity of AEs, although the doses used may not have been comparable. Headache, insomnia and somnolence were most commonly reported on placebo; fatigue, insomnia and headache on venlafaxine, and headache and dizziness on pregabalin. All AEs were followed up, no adverse sequelae were reported, and there were no serious AEs.

Thirty-four participants took 57 concomitant medications during the study. Thirty-one medications were taken to alleviate adverse events (e.g. paracetamol, mouth ulcer treatment), two were local anaesthetic for dental work, nine were for pre-existing conditions (e.g. vitamin supplements) and 15 were taken for prophylactic reasons (e.g. multivitamins, contraceptives). None were judged to interfere with the study measures.

Discussion

This is the first study to investigate the effects of venlafaxine and pregabalin using an experimental model of GAD and panic anxiety symptoms in healthy volunteers. This was a study of 54 healthy volunteers monitored and assessed over a 25 day period. The inhalation of 7.5% CO_2 for 20 minutes and the inhalation of 35% CO_2 as a single breath, vital capacity inhalation robustly increased subjective measures of panic and anxiety. Both CO_2 inhalations increased ratings of feeling fearful, anxious, like leaving the room, stressed, tense, nervous and worried, and decreased ratings of feeling relaxed and happy. The 7.5% CO_2 inhalation increased ratings of alertness and irritability compared with air. The 35% CO_2 inhalation increased anticipatory state anxiety compared with the other inhalations. It is thought this was due to the anticipation of the higher concentration of CO_2. However, as the presentation of air and 7.5% CO_2 inhalations was not counterbalanced, a hangover of the 7.5% CO_2 inhalation effects cannot be ruled out. A limitation of this study is a lack of baseline measures taken before each inhalation; however, another study using the same procedure performed by our group found Visual Analogue Scales of anxiety returned to baseline levels within 10 minutes after the 7.5% CO_2 inhalation (Bailey et al., 2009). Physiological analysis of the CO_2 inhalations showed both concentrations increased systolic and diastolic blood pressure, and heart rate. These findings replicate our previous CO_2 inhalation studies (Bailey, 2003, 2005, 2007, 2009; Papadopoulos et al., 2010; Seddon et al., 2010).

No significant effects of drug treatment on CO_2 responses were found. There were trends towards reduced alertness generally in the venlafaxine group compared with the pregabalin group, and PSI scores of panic were lower in the pregabalin group at the peak of the 35% CO_2 rating compared with the venlafaxine group. Ratings of feeling tense and nervous were non-significantly lower in the venlafaxine and pregabalin groups compared with placebo at the peak of the 7.5% CO_2 inhalation. Systolic and diastolic blood pressure and heart rate were significantly higher in the venlafaxine group compared with both the pregabalin and placebo group at weekly health checks, and on the CO_2 challenge days regardless of inhalation, which is probably due to the noradrenergic effects of venlafaxine. In a study by Hood et al. (2010) of a clonidine challenge in 10 untreated GAD patients, seven venlafaxine treated GAD patients and seven controls showed no effect of venlafaxine treatment on clonidine sensitivity. The authors concluded that the noradrenergic effects of venlafaxine were not pivotal in reducing anxiety, which may explain why, in our study, there was no significant change in psychological response to venlafaxine in line with the observed change in physiological response.

Based on previous studies, Bailey and Nutt (2008) propose that CO_2 inhalation can elicit anxiety responses by reducing the amount of available GABA, and that this may occur alongside noradrenergic and serotonergic activation. A single dose of lorazepam (a benzodiazepine agonist) significantly reduced CO_2-induced feelings of fear, feeling like leaving the room, tension and worry (Bailey et al., 2007). Twenty-one days' dosing of paroxetine (a SSRI), however, only significantly reduced CO_2-induced feelings of nervousness (Bailey et al., 2007). Reasons suggested for this were the small number of participants, and perhaps insensitive scales. Because of this, the current study included 18 participants in each group, and also used a scale based on the DSM-IV and the International Classification of Diseases version 10 (ICD-10) criteria for GAD-C, but still drug attenuation of the 7.5% and 35% CO_2 challenge did not reach significance. Another reason for a lack of significant effect may have been inter-individual variability. Although balanced for demographic characteristics, other factors, such as timing of cigarettes smoked, may affect response to the CO_2 challenge. Attwood et al. (2009) found non-abstinent smokers reported reduced anxiety in the 7.5% CO_2 challenge compared with abstinent smokers, with non-smokers positioned in between. All smokers were restricted from smoking for 3.5 hours before inhalations, but only 1.5 hours of this time was under observation in the research facility. Also, the anxiety sensitivity and state anxiety of participants on test days were slightly lower than normal values would suggest, which may have biased responses. Notably, participant scores for extraversion were higher and scores for neuroticism and harm avoidance were lower than normal values, which is to be expected in individuals who volunteer for medical trials and in line with our previous studies (Bailey and Nutt, 2001, 2005). Compliance was thought to be good, but most doses were not witnessed. However, physiological measures taken during weekly health checks showed blood pressure and heart rate were significantly higher in the venlafaxine group compared with pregabalin and placebo (data not shown), indicating general compliance.

A study by Papadopoulos et al. (2010) showed no significant subjective effects of acute doses of the anxiolytic drugs propranolol, hydroxyzine or flupentixol on the 7.5% CO_2 challenge when compared with placebo. The authors suggest this may have been due to propranolol being more effective for somatic symptoms rather than for psychological symptoms (File and Lister, 1985) and the doses of hydroxyzine (an antihistamine; H1 receptor agonist) and flupentixol (an antipsychotic; dopamine D1 and D2 receptor antagonist) being sub-clinical (to minimise side effects). However, a recent study by Bailey et al. (2011) found seven days'

dosing of a corticotropin-releasing factor antagonist (R317573) significantly reduced subjective panic and anxiety measures during the 7.5% CO_2 inhalation compared with placebo, but not quite to the extent of a single dose of lorazepam. Comparing different anxiolytics with fast and slower onset of action is difficult, as there may not be an equivalence of doses.

Rather than a difference in effect between acute or chronic dosing, an alternative postulation would be that a significant influence on the CO_2 challenge may only be elicited by benzodiazepines or similarly potent anxiolytics acting on the $GABA_A$ receptor. Indeed, several patient studies (but not all) have shown that a single dose of alprazolam (Pols et al., 1996; Sanderson et al., 1994) and clonazepam (Nardi et al., 2000; Valença et al., 2000) can attenuate the anxiety response to 35% CO_2. Valença et al. (2002) have also demonstrated this effect after six weeks of treatment with clonazepam. A study by Zwanzger et al. (2003) showed that a single dose of alprazolam can attenuate CCK-4 provoked panic in healthy volunteers. A recent study by Bailey et al. (2009) of the acute effects of zolpidem (5 mg), alprazolam (1 mg) and placebo showed alprazolam (a benzodiazepine) significantly reduced ratings of fear, panic and generalised anxiety compared with placebo during the 7.5% CO_2 challenge, and reduced panic and generalised anxiety scores during the 35% CO_2 challenge. Zolpidem (an agonist with preference for alpha-1 subtype binding in the $GABA_A$ receptor) significantly reduced feeling like leaving the room, and feeling worried and stressed compared with placebo during the 7.5% CO_2 challenge.

Although venlafaxine and pregabalin at 150mg and 200mg respectively have been shown to have anxiolytic properties in GAD patients (Davidson et al., 1999; Pohl et al., 2005), it appears that they may not provide a sufficiently potent anxiolytic effect for the CO_2 challenge model, or the CO_2 models were not mechanistically similar enough to GAD. Either way, 21 days' tapering up to these clinically effective doses was not shown to consistently attenuate the anxiogenic effects of hypercapnic gas, although trends towards attenuation were noted. It is possible that the doses of the drugs used in this study were not high enough to elicit significant effects on the CO_2 challenges. This is unlikely with respect to venlafaxine as it is licensed for anxiety at the dose range of 75mg–225mg daily, but may be the case for pregabalin, given that up to 600mg daily is permitted. We did not progress to this dose on account of tolerability concerns, although no participants withdrew from the pregabalin group and this appears to have been groundless. A limitation to this study was the lack of use of a benzodiazepine for direct comparison, which may have resolved some issues such as equivalent dosing.

One consideration is that of differences between the modes of action of the anxiolytics that the CO_2 challenge may be exposing. Benzodiazepines have been shown in most (but not all) GAD patient studies to be more efficacious at alleviating somatic anxiety rather than psychic anxiety (e.g. Rickels et al., 1988, 1993). In contrast, pregabalin (after 4–6 weeks (Lydiard et al., 2010)) and venlafaxine (after eight weeks (Stahl et al., 2007); eight weeks and six months (Meoni et al., 2004)) have been shown to be more efficacious at alleviating psychic than somatic anxiety in patients with GAD. The PSI includes both somatic and psychic questions, and analysis of these subdivisions showed that those on placebo reported a trend towards a larger increase in somatic symptoms compared with psychic symptoms when inhaling 7.5% CO_2. This may be an important distinction when developing novel anxiolytics, as somatic symptoms can often be the primary complaint of anxious patients (Meoni et al., 2004). The patients' perception of the improvement in the somatic symptoms of anxiety can provide a considerable contribution to their perception of treatment efficacy, so much so that Meoni et al. (2004) suggest the improvement of somatic anxiety should be regarded as an essential requirement of any anxiolytic medication.

To conclude, this study showed that venlafaxine and pregabalin had no significant effect on either CO_2 challenge model in healthy volunteers. This may have been due to some classes of anxiolytics, depending on their mode of action or speed of onset, mediating these effects more than others. Further research is required into the emerging distinctions between anxiolytics found when administered for CO_2-induced anxiety, or the use of other techniques, such as cognitive tasks, to separate the expressions of anxiety elicited during the CO_2 challenges.

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Conflict of interest

AD, VO-H, ASR and JEB have no conflicts of interest to declare. GRD, CTD and KC are directors and shareholders in P1vital Limited. DJN has provided consultancy services to Pfizer, GSK, Novartis, Organon, Cypress, Lilly, Janssen, Lundbeck, BMS, Astra Zeneca, Servier, Hythiam and Sepracor, received honoraria from Wyeth, Reckitt-Benkiser and Cephalon, received grants or clinical trials payments from MSD, GSK, Novartis, Servier, Janssen, Lundbeck, Pfizer, Wyeth and Organon and holds shares in GSK.

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