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The effect of a clinically effective and non-effective dose of lorazepam on 7.5% CO_2-induced anxiety

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Symptoms of anxiety induced by 7.5% CO_2 inhalation can be attenuated by acute administration of $GABA_A$ receptor anxiolytics such as lorazepam and alprazolam. This study investigated if these effects are dose-related, by comparing a 0.5 mg dose (considered non-clinically effective) and a 2 mg dose of lorazepam (clinically effective) on 7.5% CO_2 inhalation.

Eighteen healthy males (mean age 20.6 years, *SD* 1.29), judged physically and mentally fit, attended three visits, each one week apart, to take each treatment in a randomised double-blind crossover design. Drugs were given 60 min prior to 20 min air inhalation, followed by 20 min 7.5% CO_2 inhalation. The order of gas presentation was single blind. Subjective ratings using visual analogue scales (VAS) and questionnaires were recorded before and after each inhalation. Blood pressure (BP), heart rate (HR), respiration rate (RR) and expired CO_2 were recorded during each inhalation.

Inhalation of 7.5% CO_2 significantly raised BP, HR, RR and expired CO_2. Ratings of feeling like leaving the room were significantly lower on 2 mg compared with 0.5 mg and placebo, and dose-dependent trends were seen in scores for VAS fearful, anxious, stressed, tense, and worried. Results may be indicative of dose-dependent effects of lorazepam in a CO_2 model of anxiety. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—carbon dioxide; anxiety; panic; lorazepam; dose-dependent; experimental human model

INTRODUCTION

The inhalation of CO_2 has been shown by several laboratories to elicit feelings of anxiety and panic and result in physiological changes such as an increase in blood pressure, heart rate and skin conductance (Gorman *et al*., 1988; Bailey *et al*., 2005; Poma *et al*., 2005; van Duinen *et al*., 2005). There is some evidence for a link between these effects (Argyropoulos *et al*., 2002; Bailey *et al*., 2003; Kaye *et al*., 2004), and several theories have been put forward as to the mechanisms involved. Cognitive theories suggest the psychological effects of CO_2 inhalation arise from a misinterpretation of physical symptoms (Holt and Andrews, 1989) or loss of control (Sanderson *et al*., 1989). Another theory suggests panic arises from a 'suffocation alarm', as a build-up of brain CO_2 levels can signal impending suffocation (Klein, 1993). Our group and others have implicated noradrenergic mechanisms of CO_2 action via the locus coeruleus, driven by medullary chemoreceptors in the carotid body, specifically gamma-aminobutyric acid (GABA) -ergic receptors (Bailey *et al*., 2003; Zhang *et al*., 2003). Recent research implicates the amygdala as a pH sensitive chemosensor (Ziemann *et al*., 2009), which triggers a behavioural response to CO_2, possibly via connections to the hypothalamus and brainstem (Gorman *et al*., 2000).

It has long been known that fear and other emotional states are moderated at least in part by GABA transmission (Biggio *et al*., 1990; Coplan and Lydiard, 1998) within the amygdala and beyond, centrally and peripherally (Bailey and Nutt, 2008; Ziemann *et al*., 2009). CO_2 inhalation results in a rapid inhibition of the function of GABA receptor-coupled chloride channels (Sanna *et al*., 1992; Concas *et al*., 1993). Agonists of GABA benzodiazepine receptors have anxiolytic effects (for example, Corbett *et al*., 1991), and abnormalities of benzodiazepine receptors have been found in panic disorder, generalised anxiety disorder (GAD) and post-traumatic stress disorder (Tiihonen *et al*., 1997; Bell

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and Nutt, 1998; Malizia *et al*., 1998; Bremner *et al*., 2000). More recently, drug development has mainly focussed on partial agonists and subtype selective $GABA_A$ modulators, for example, the development of TPA023, an alpha-2/alpha-3 agonist (Atack, 2009).

Developing an experimental model of anxiety in healthy volunteers is useful to determine the various parameters that may affect the efficacy of any anxiolytic drug, and may assist drug development prior to studies in patient populations. The CO_2 model of anxiety is therefore an attractive prospect for testing early-phase efficacy and proof of concept (Bailey *et al*., 2011a). In developing and validating the CO_2 model, we have found that enhancing $GABA_A$ receptor function with drugs that act as agonists at the benzodiazepine binding site on the receptor, such as lorazepam and alprazolam, significantly reduced ratings of panic and anxiety compared with placebo during 7.5% and 35% CO_2 challenges in healthy volunteers (Bailey *et al*., 2007, 2009). Similar results have been shown in patients with panic disorder after 1 week of paroxetine, and to a lesser extent, reboxetine (Perna *et al*., 2004), and other tricyclic antidepressants and selective serotonin reuptake inhibitors (Gorman *et al*., 1997; Perna *et al*., 2002).

Acute administration of lorazepam has been found to reduce CO_2-induced anxiety in healthy volunteers and has been successfully used as a positive comparator in a recent CO_2 study of a new corticotropin-releasing factor antagonist (Bailey *et al*., 2011b). Over a longer period, lorazepam is also effective in reducing some of the symptoms of GAD, perhaps indicating in turn that the CO_2 challenge may be a good model in healthy volunteers for GAD. Feltner *et al*. (2003) and Laakmann *et al*. (1998) respectively gave 68 GAD patients 6 mg doses and 47 GAD patients 3 mg doses of lorazepam for 4 weeks and found significant reductions in the Hamilton Anxiety Scale scores (and State Trait Anxiety Inventory [STAI] scores in the latter study) compared with placebo. Cutler *et al*. (1993) also used lorazepam as a positive control and found that 4 weeks' dosing of 2 to 6 mg of lorazepam significantly reduced HAM-A scores in 89 GAD outpatients. It may be that a reduction in GAD would only be seen after chronic dosing; however, acute doses may change cognition prior to changes in mood. For example, Harmer *et al*. (2009) showed that a single dose of reboxetine altered negative affect bias in patients with depression.

The recommended prescription dose of lorazepam for anxiety is 1–4 mg daily (British National Formulary, 2010). Healthy volunteer studies have shown that lorazepam is effective at a dose of 2 mg, but not below. For example, under the threat of electric shock, Schunck *et al*. (2010) found that 1 mg lorazepam did not significantly reduce reported anticipatory anxiety compared with placebo, despite reduced activity (fMRI changes) in brain areas associated with anxiety circuitry. Also, a study of the fear-potentiated startle reflex showed that anticipatory anxiety was attenuated by 2 mg but not 1 mg of lorazepam (Graham *et al*., 2005).

Many studies have been performed to assess the effects of different types of drugs on the CO_2 challenge, but none have sought to determine the effects of different doses of the same drug in this model. The identification of a minimum effective dose for an anxiolytic effect in the 7.5% CO_2 inhalation model may help to determine the doses of new anxiolytic medications that may be potentially efficacious. This in turn may be valuable in determining the doses for subsequent patient efficacy studies. Thus, combining early-phase dose-escalation studies with the CO_2 challenge may be a more cost-effective and faster method of drug development. To investigate dose-dependent effects on the CO_2 challenge, and thus enable comment on its usefulness as a drug development tool in healthy volunteers, we present a new study comparing the effects of a clinically effective (2 mg) and non-effective (0.5 mg) dose of lorazepam on CO_2-induced symptoms of anxiety. The dose of 2 mg was chosen as a 'clinically effective' dose as has been used successfully in patient studies (e.g. Cutler *et al*., 1993), and Bailey *et al*. (2007) found acute effects of 2 mg lorazepam on CO_2-induced anxiety. Non-significant attenuation was found after 1 mg lorazepam (Nutt and Bailey, 2002); therefore, it was hypothesised that a lower dose may have no effect. The dose of 0.5 mg was also chosen as it is half the recommended starting dose for patients being treated with lorazepam. We hypothesised that the clinically effective 2 mg dose would significantly reduce CO_2-induced anxiety-related symptoms, whereas the 0.5 mg dose would not.

METHODS

Ethical considerations

The study protocol was approved by the Central and South Bristol Research Ethics Committee, local NHS Trusts and the Medicines and Healthcare products Regulatory Agency, and performed in accordance with ICH Good Clinical Practice and the Declaration of Helsinki, 2008.

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. We were contacted by 99 volunteers, of

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which 89 requested further information and received the participant information sheet.

Prior to inclusion, participants attended a screening visit where they were informed of study procedures and gave signed informed consent. A physical examination was performed by a study physician, including an electrocardiogram, and medical histories were taken. Participants were interviewed to ensure no history of significant psychiatric disorder, such as depression, anxiety or obsessive-compulsive disorder, or first-degree relatives with severe anxiety or panic disorder. Other exclusion criteria were as follows: 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 previous participation in a CO_2 study. A urine sample was provided by participants to screen for drugs of abuse. Neither concomitant medications nor intake of any medication (apart from over-the-counter remedies deemed not to interfere with study procedures) were allowed for 2 weeks prior to testing. Participants' general practitioners (family doctors) were informed of the study, and all participants were reimbursed £100 for their time. Thirty-two volunteers attended a screening, where twenty eight passed and four failed because of a personal or family history of psychiatric problems. Ten dropped out before the study start because of personal reasons and concern over the time commitment.

Study design and procedure

This was a double blind placebo-controlled, within-subject, three-period crossover design with 18 participants to achieve a balanced order-effect design. Each of the three test days was a week apart, on consecutive weeks.

On arrival for each test day, the investigator asked questions to determine that the participants were still fit and willing to undergo the study procedure, and that their health and medication status had not changed since screening. A breathalyzer recording was performed to ensure that the participants had not recently consumed alcohol, and urine samples were provided to check for use of drugs of abuse.

Study medication was checked and given 60 min before the first gas inhalation period according to the randomisation schedule, and approximately 50 min later, participants were settled into a comfortable chair in the testing room for the test period. Each test period consisted of an inhalation of air for 20 min, 10 min rest, an inhalation of CO_2 7.5% for 20 min and finally 10 min of rest. Subjective ratings of the participants' emotional state were recorded 10 min before the first gas inhalation and directly after each gas inhalation, where the participants were asked to rate how they felt at the peak of their experience during each inhalation. Heart rate and blood pressure were measured throughout the two gas inhalation periods. One minute prior to the end of each gas inhalation period, expired CO_2 and respiratory rate were recorded.

An hour after the testing period had finished, the participants were allowed to leave the clinic if they felt well, and were instructed not to drive, use machinery, participate in any potentially hazardous activity or consume alcohol on the day of drug administration, or longer, if they felt affected by the drug.

Study drugs

Lorazepam 0.5 and 2 mg, and placebo were prepared, randomised and packaged by University Hospitals Bristol NHS Foundation Trust Pharmacy. The study drugs were prepared in matching blue capsules in individual containers labelled 'Period 1', 'Period 2' and 'Period 3'. The study drugs were administered under the supervision of blinded researchers on site to non-fasted participants with approximately 200 ml of non-carbonated water, 60 min prior to the beginning of the first gas inhalation (air inhalation). There was a washout period of at least one week between each of the three treatment periods.

Delivery of gas

The gas mixture used was CO_2 7.5%/O_2 21%/N 71.5% or Medical Air, delivered via a nasal–oral exercise facemask (Hans Rudolph, Shawnee, KS) attached to a 500-L Douglas bag via tubing. At least two investigators attended the sessions, and one investigator remained in sight of the participants throughout the procedure. Participants were seated comfortably during inhalations, and 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 participants 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.

Subjective measures

Participants scored visual analogue scales (VAS) verbally on a scale of 0 (*not at all*) to 100 (*the most ever*) by using adjectives previously used by our group (e.g. Bailey *et al*., 2007): alert, fearful, relaxed, anxious, happy, feel like leaving, paralysed, stressed, tense, nervous, irritable and worried. VAS ratings were performed prior to dosing and at baseline (before the first inhalation), and at the end of each gas inhalation

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(+20 min) and 10 min after the end of the inhalation (+30 min). 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 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* and 4 = *very severe*. The PSI was adapted from Clark and Hemsley (1982), and lists 34 items and has been used in studies of panic provocation (Nutt *et al*., 1990; Bell *et al*., 2002) and previous CO_2 studies (Argyropoulos *et al*., 2002; Bailey *et al*., 2005, 2007). It was administered prior to dosing, at baseline (before the first inhalation), and at the end of each gas inhalation (+20 min).

The Generalised Anxiety Disorder Criteria Inventory (GAD-C) is based on the World Health Organisation (WHO) International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) criteria for GAD, and was used to measure participants' current anxiety state. This was administered at the same time points as the PSI.

The Spielberger State Trait Anxiety Inventory—State (SSAI; Spielberger, 1983) was used to measure state anxiety and was administered prior to dosing, at baseline (before the first inhalation), and 10 min after the end of each inhalation period.

The Anxiety Sensitivity Index (ASI; Reiss *et al*., 1986) is a scale of 16 statements, listing possible negative aspects of anxiety, such as additional anxiety or fear, illness, embarrassment and loss of control. Examples of these statements are 'It is important to me to stay in control of my emotions' and 'Other people notice when I feel shaky'. Participants were required to rate the intensity of each statement as *very little*, *a little*, *some*, *much* or *very much*. The ASI was used alongside the trait section of the Spielberger STAI (Spielberger, 1983), both as measures of trait anxiety.

Objective measures

Continuous measures of blood pressure and heart rate were obtained 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 artefacts, the participants were instructed to stay still and not to move their hand, or cross their legs. Data were captured onto a MS-DOS-based program (Microsoft Corp., Washington, USA) and later analysed using software designed for the purpose.

A measure of expired CO_2 and respiratory rate were recorded 19 min into each inhalation, via a capnometer and nasal cannulae put in place before the start of each inhalation. A time point prior to the end of inhalation was chosen so the participants would be stable in their management of breathing the CO_2.

Power calculation. The power calculation was based on a within-subjects study of the effects of lorazepam 2 mg and placebo on the 7.5% CO_2 inhalation (Bailey *et al*., 2007). Two independent populations with mean VAS 'anxiety' at peak effects of CO_2 of 31.3 (placebo) and 17.9 (lorazepam, 2 mg), with a common standard deviation of 12.2, suggested that to detect a significant difference at the alpha = 0.05 level, an *N* of 13 would be required for this study. This number was increased to 18 to allow for variation in parameters between experiments and to balance the randomisation.

RESULTS

Participants

Eighteen male participants were recruited, age range 19–39 years (mean 20.6 years, *SD* 1.29). There were no discontinuations; however, the data from one participant were excluded from the statistical analyses because of a positive urine sample for benzodiazepines before dosing on both days 2 and 3 of the study. The participant denied concomitant drug use, but endogenous benzodiazepine presence or hangover from the previous session (each a week apart) were unlikely.

One participant was an occasional smoker (<5 items per day), and the other participants reported they were non-smokers. All were judged to have normal trait anxiety levels but possibly lowered anxiety sensitivity, as measured by the trait part of the STAI (mean 33.1, *SD* 5.36) and the ASI (mean 12.9, *SD* 5.37). For comparison, the average trait anxiety score for men and women aged 19 to 39 years is 35.9 (*SD* 9.65; Spielberger, 1983), and the average ASI score is 19.1 (*SD* 9.11; Peterson and Reiss, 1993). None were taking concomitant medication before the study.

Analyses

Comparisons of peak gas effects (peak air vs peak 7.5% CO_2) were made for subjective variables. Blood pressure and heart rate were averaged over the 20 min inhalations and compared. Respiratory rate and expired CO_2 measures were taken 1 min prior to the end of each inhalation and compared. Subjective and objective variables were analysed using individual repeated measures ANOVAs (and VAS scales were also analysed using multivariate ANOVA) for Drug, Gas and Drug * Gas interactions using the Greenhouse–Geisser correction. Although not all VAS variables were

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normally distributed, it is assumed that the test was robust enough to withstand such violations. Post hoc analyses were simple main effects and paired *t* tests. All data were analysed using SPSS (IBM Corp., New York, USA) version 16.0 for Windows.

Paired *t* tests comparing baseline levels before each inhalation showed no significant differences for the majority of subjective measures. Two exceptions to this, where significant decreases were found in the CO_2 baseline compared with those in the air baseline, were the ratings of feeling alert on 2 mg lorazepam ($t[16] = 2.1$, $p < 0.05$) and feeling stressed on 0.5 mg lorazepam ($t[16] = 2.3$, $p < 0.05$).

Order effects

The data were examined for order effects using one-way ANOVAs and Tukey's for post hoc analyses. Some order effects were noted. Participants felt significantly more relaxed ($F[2,17] = 5.1$, $p < 0.05$) and less tense ($F[2,17] = 5.0$, $p < 0.05$) during the CO_2 inhalation on 2 mg lorazepam on day 3 compared with day 1. It is possible that this was due to an expectation of the potential effects of a higher dose of lorazepam, indicating it was possible the participants thought that they probably had the 0.5 mg dose or placebo on the preceding two study days. Anecdotally, the majority of participants thought they knew when they had taken 2 mg lorazepam, but could not detect the difference between the 0.5 mg dose and placebo. Participants were also significantly more nervous on placebo during their first inhalation on day 1 compared with day 3 ($F[2,17] = 5.4$, $p < 0.05$). This was probably because they lacked experience of the experimental scenario, and this nervousness may have been attenuated by lorazepam. Lastly, participants were more irritable on day 3 during the CO_2 inhalation when on placebo, compared with day 1 ($F[2,17] = 3.8$, $p < 0.05$). This was the last inhalation of the study, and without lorazepam to attenuate mood, participants may have become irritable when wishing the study to finish.

Questionnaires

Compared with air, the inhalation of 7.5% CO_2 significantly increased the total PSI scores ($F[1,17] = 21.4$, $p < 0.001$), GAD-C scores ($F[1,17] = 18.4$, $p < 0.01$) and SSAI scores ($F[1,17] = 10.9$, $p < 0.01$). These effects of 7.5% CO_2 were not attenuated by either dose of lorazepam.

Visual analogue scales

There was a significant drug effect on ratings of feeling alert ($F[2,17] = 4.7$, $p < 0.05$), whereby participants felt less alert on 2 mg lorazepam compared with 0.5 mg lorazepam ($t[16] = 2.2$, $p < 0.05$) and placebo ($t[16] = 3.3$, $p < 0.01$) during the air inhalation only. This effect was not apparent in the more alerting CO_2 condition, despite an overall decrease in alertness on 2 mg lorazepam as the test session progressed (see Analyses section).

There was a significant drug effect on ratings of feeling like leaving the room ($F[2,17] = 4.7$, $p < 0.05$), whereby participants felt less like leaving on 2 mg lorazepam compared with placebo ($t[16] = 2.3$, $p < 0.05$), for the 7.5% CO_2 inhalation only. The 2 mg dose of lorazepam may have reduced the desire for escape during the CO_2 inhalation, which was not seen using the sub-clinical dose of 0.5 mg lorazepam (see Figure 1).

During the inhalation of 7.5% CO_2 compared with air, participants reported increased ratings of feeling: alert ($F[1,17] = 9.1$, $p < 0.01$), like leaving the room ($F[1,17] = 18.0$, $p < 0.01$), fearful ($F[1,17] = 15.6$, $p < 0.01$), anxious ($F[1,17] = 18.7$, $p < 0.01$), paralysed ($F[1,17] = 4.9$, $p < 0.05$), stressed ($F[1,17] = 21.3$, $p < 0.001$), tense ($F[1,17] = 19.5$, $p < 0.001$), nervous ($F[1,17] = 14.4$, $p < 0.01$), irritable ($F[1,17] = 8.9$, $p < 0.01$) and worried ($F[1,17] = 15.4$, $p < 0.01$). During the inhalation of 7.5% CO_2, compared with air, participants reported decreased ratings of feeling relaxed ($F[1,17] = 45.0$, $p < 0.001$) and happy ($F[1,17] = 41.8$, $p < 0.001$; see Table 1).

A multivariate ANOVA (Wilks' lambda) of all VAS ratings showed that the multivariate main effect for drug was not significant ($F[24,42] = 1.5$, $p = 0.137$), but confirmed that there was a significant effect of inhalation ($F[24,42] = 7.5$, $p < 0.001$).

Physiological measures

There was a significant drug effect on heart rate ($F[2,17] = 8.3$, $p < 0.01$), whereby heart rate on placebo was significantly lower compared with 2 mg lorazepam ($p < 0.01$). There was a significant interaction between drug and gas conditions ($F[2,17] = 4.9$, $p < 0.05$). Heart rate was significantly higher on 2 mg lorazepam compared with placebo during the air inhalation ($t[16] = 2.9$, $p < 0.05$), and a significantly higher on 2 mg lorazepam compared with 0.5 mg lorazepam ($t[16] = 2.9$, $p < 0.05$) and placebo ($t[16] = 5.1$, $p < 0.001$) during the CO_2 inhalation.

Compared with the air inhalation, the 7.5% CO_2 inhalation significantly increased the following: respiratory rate ($F[1,17] = 38.8$, $p < 0.001$), expired CO_2 ($F[1,17] = 80.0$, $p < 0.001$), systolic blood pressure ($F[1,17] = 43.4$, $p < 0.001$), diastolic blood pressure ($F[1,17] = 16.6$, $p < 0.01$) and heart rate ($F[1,17] = 30.7$, $p < 0.001$; see Table 1).

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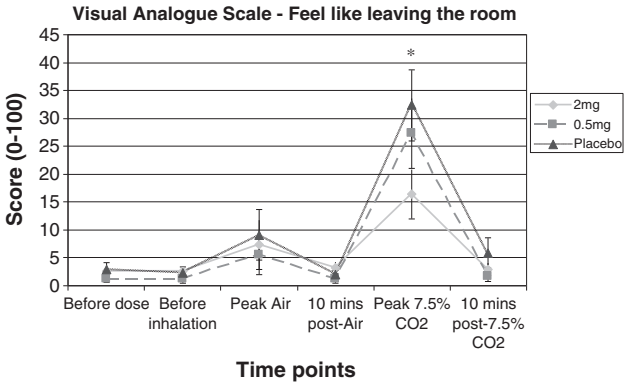

Figure 1. VAS ratings for 'feeling like leaving the room' were significantly lower with 2 mg lorazepam compared with 0.5 mg lorazepam and placebo at peak during the 7.5% CO_2 inhalation (*$ps < 0.05$, $n = 17$). Although not statistically significant, ratings of feeling fearful, anxious, stressed, tense and worried showed similar dose-dependent trend patterns (2 mg < 0.5 mg < placebo)

Adverse events and concomitant medications

In total, 41 adverse events (AEs) were reported after study consent by 16 participants. The most common AEs were somnolence, dizziness, fatigue, feeling abnormal and coordination abnormal on lorazepam 2 mg; headache and dizziness on lorazepam 0.5 mg; and somnolence, headache, muscle weakness and visual impairment on placebo. Where appropriate, participants were escorted to a taxi for the journey home. All were followed up the day after each study day, and no adverse sequelae were reported. Two participants used concomitant medication during the study period: a single 1 g dose of paracetamol for a headache and a spoonful of over-the-counter cough syrup for a transient sore throat.

DISCUSSION

This study investigated the effects of clinically effective and non-clinically effective doses of lorazepam on the inhalation of 7.5% CO_2. The aim was to determine if the anxiolytic effects of lorazepam on the CO_2 challenge were dose-dependent, and if the CO_2 challenge was sensitive to sub-clinical doses of lorazepam (as measured by significant differences in subjective reports and objective physiological measurements).

Compared with the inhalation of air, the inhalation of 7.5% CO_2 for 20 min significantly increased subjective ratings of panic and anxiety, alertness, fear, paralysis, stress, tension, nervousness, irritability, worry and feeling like leaving the room, and increased objective respiration rate, expired CO_2, systolic and diastolic blood pressure, and heart rate. The CO_2 inhalation also decreased feelings of being relaxed and happy. These

Table 1. Inhalations of 7.5% CO_2 significantly increased negative subjective symptoms and cardiovascular measures, and reduced positive subjective symptoms, compared with inhalations of air

	At peak/during air inhalation			At peak/during 7.5% CO_2 inhalation		
Variable	2 mg lorazepam	0.5 mg lorazepam	Placebo	2 mg lorazepam	0.5 mg lorazepam	Placebo
VAS alert	36.5 (18.94)	49.4 (20.76)*	54.1 (20.56)**	51.8 (28.83)	58.5 (19.90)	64.4 (21.13)
VAS fearful	5.0 (8.10)	5.0 (7.29)	7.1 (9.36)	15.0 (17.94)	19.1 (19.06)	25.6 (23.58)
VAS relaxed	70.9 (21.08)	72.1 (13.47)	66.2 (17.99)	39.7 (20.19)	32.9 (23.79)	37.9 (20.47)
VAS anxious	6.2 (11.11)	10.0 (13.58)	10.6 (14.02)	23.8 (21.40)	30.0 (22.36)	31.8 (27.04)
VAS happy	60.9 (17.07)	64.1 (16.32)	63.2 (14.46)	42.6 (18.47)	37.4 (20.70)	42.4 (21.15)
VAS leaving	7.4 (18.38)	5.6 (14.78)	9.1 (18.48)	16.5 (18.44)	27.4 (25.93)	32.4 (26.23)*
VAS paralysed	2.9 (9.69)	1.5 (3.86)	0.9 (2.64)	7.9 (17.14)	4.4 (8.99)	4.4 (8.64)
VAS stressed	5.9 (15.83)	4.4 (6.82)	7.1 (11.33)	23.2 (28.28)	24.1 (21.88)	32.4 (26.58)
VAS tense	5.0 (9.52)	5.9 (8.15)	7.1 (9.69)	25.0 (28.56)	24.7 (21.03)	30.3 (25.95)
VAS nervous	7.1 (13.81)	5.3 (8.00)	8.5 (11.56)	16.2 (20.27)	22.6 (20.40)	21.8 (22.98)
VAS irritable	6.2 (16.06)	2.9 (7.72)	4.7 (8.19)	15.0 (21.43)	18.8 (20.50)	19.4 (24.17)
VAS worried	4.7 (10.07)	5.3 (8.00)	8.8 (10.39)	15.6 (18.10)	20.6 (21.06)	22.4 (22.44)
PSI	8.0 (5.63)	6.7 (5.42)	6.4 (1.18)	18.5 (13.68)	16.2 (11.00)	14.8 (3.23)
GAD-C	5.3 (4.09)	4.7 (0.86)	3.7 (1.98)	10.1 (6.92)	8.5 (2.25)	6.9 (3.39)
SSAI[a]	31.4 (7.65)	19.5 (16.79)	18.8 (9.79)	35.0 (10.53)	22.8 (17.30)	21.4 (10.36)
RR	13.1 (3.60)	11.6 (3.08)	11.3 (3.02)	18.4 (4.06)	18.8 (6.12)	17.2 (4.45)
ExpCO_2	5.5 (0.73)	5.7 (1.30)	5.2 (0.74)	7.2 (0.95)	7.5 (0.56)	7.4 (0.63)
SBP	124.4 (19.86)	124.5 (15.69)	126.9 (13.63)	134.9 (22.10)	136.6 (19.53)	141.0 (17.04)
DBP	79.2 (14.35)	78.9 (11.93)	78.7 (9.75)	84.1 (14.30)	83.4 (13.69)	85.9 (13.43)
HR	73.7 (7.15)	71.3 (7.04)	70.3* (6.64)	78.8 (7.84)	74.8 (7.36)*	72.4 (7.29)**

Table shows mean scores with standard deviations in parentheses ($n = 17$). VAS, visual analogue scale; PSI, Panic Symptom Inventory; GAD-C, Generalised Anxiety Disorder Criteria Inventory; SSAI, Spielberger State Trait Anxiety Inventory (State); RR, respiratory rate (breaths/min); ExpCO_2, % expired CO_2; SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); HR, heart rate (bpm).
[a]Measures taken 10 min after inhalation, not at peak.
*$p < 0.05$ compared with 2 mg.
**$p < 0.01$ compared with 2 mg.

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effects were expected and replicate the findings of our previous CO_2 inhalation studies (e.g. Bailey *et al.*, 2005, 2007, 2009, 2011b; Papadopoulos *et al.*, 2010; Diaper *et al.*, 2012).

The administration of lorazepam did not significantly affect blood pressure or respiration. There were few notable dose-dependent effects, and the study failed to replicate the results of Bailey *et al.* (2007). During the air inhalation, ratings of alertness were significantly lower under the 2 mg dose of lorazepam compared with the 0.5 mg dose and placebo, as expected. This is a likely indicator that the 2 mg dose had a stronger sedative effect than the 0.5 mg dose or placebo, and the 7.5% CO_2 inhalation was sufficiently arousing and/or anxiogenic to negate these effects. At the peak effect of the CO_2 inhalation, participants on the 2 mg dose reported significantly lower ratings of feeling like leaving the room than participants taking the 0.5 mg dose and placebo. This pattern is also present for ratings of feeling fearful, anxious, nervous, irritable and worried. Although differences were not significant, for all of these measures, the anxiogenic effects of the CO_2 inhalation were lowest on the 2 mg dose, followed by the 0.5 mg dose, and highest on placebo. As there were numerous statistical tests used to analyse the data, it is possible that the significant change in the variable of 'leaving the room' was spurious, particularly as the MANOVA performed on the VAS ratings did not reach significance for drug effect.

These results indicate that there is little evidence for a dose-related effect of lorazepam in the 7.5% CO_2 challenge. Trends present in the data, which would have supported this evidence, may not have reached significance for several reasons. Firstly, the number of participants in this study, although maximised by using a crossover design, was small. With more data to reduce variability and increase power, differences in the data may have reached significance. Participants may have had a lower ASI than average; this is perhaps not surprising in a sample volunteering for a clinical trial. In future studies, it may be prudent to screen for normal ASI as well as trait anxiety. In addition, the sample consisted of young men, not typical of anxiolytic users who tend to be older and female (Ohayon *et al.*, 1998), and scales such as the VAS for anxiety were created for clinical populations rather than healthy volunteers (Battaglia and Perna, 1995). However, these scales have been successfully used in our group's previous work (e.g. Bailey *et al.*, 2007).

Order effects were noted for several VAS measurements, for example, feeling more nervous generally on the first test day, and feeling more relaxed and less tense on the 2 mg dose of lorazepam on the last test day compared with the first test day. These effects may be indicative of participants becoming more comfortable with the test conditions and may have biased results and contributed to a lack of effect in these variables when comparing peak to peak effects. Indeed, Carter *et al.* (1995) found an attenuated effect in panic disorder patients given a 5.5% CO_2 inhalation when they felt safe (in the company of someone they felt safe with). However, our group (Papadopoulos *et al.*, 2010; Seddon *et al.*, 2011) and others have found the CO_2 challenge to have good test–retest reliability, so any general change in approach by the participants to our test scenario should not have affected the potency of the CO_2 challenge. Anecdotally, the majority of participants thought they knew when they had taken 2 mg lorazepam and when they had not. These predictions were not recorded, so participants may have been wrong in their judgements, and the study may have suffered confounds from a placebo or nocebo effect. Participants may have anticipated small or large doses when they thought they were due. Inspection of the data shows that respiration rate was (not significantly) higher during the air inhalation on 2 mg lorazepam compared with the 0.5 mg dose and placebo, which is unexpected for a drug with known side effects of drowsiness and respiratory depression. It is possible that the side effects of the large dose of the drug, where apparent, increased stress and respiration rate in participants (particularly during the non-alerting air inhalation), which may have confounded results.

It is possible, although unlikely, that some volunteers may not have been experiencing maximum drug effects during the CO_2 challenges. Lorazepam has a latency of approximately 1–3 h before it takes effect (Greenblatt *et al.*, 1988), but the length of time can be influenced. Although all participants were required to weigh over 60 kg for study entry, standard doses were given to participants regardless of weight. The participants' body mass index (BMI) ranged between 19.5 and 31, and 29% of participants were overweight (a BMI greater than 25). This may have delayed the time to drug onset. Also, there were no instructions given to participants regarding food, and differences in food consumption may have affected absorption and therefore affected results. For example, Erdman *et al.* (2007) found no difference in the amount of alprazolam absorbed after a high fat breakfast, but absorption was slower, delaying the onset of drug effect.

In summary, despite limitations, this study shows non-significant trends towards dose-related effects of lorazepam in the 7.5% CO_2 challenge. These results are contrary to a previous study, which found significant acute anxiolytic effects of 2 mg lorazepam on

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symptoms of anxiety induced by the 7.5% CO_2 inhalation (Bailey *et al*., 2007). This in turn does not provide support for the use of the 7.5% CO_2 inhalation as a model for GAD. However, a further study of lorazepam with a larger number of participants, perhaps with dosing over a period of weeks, may be more valid in assessing this model.

CONFLICT OF INTEREST

Alison Diaper, Andreas Papadopoulos, Ann Rich and Jayne Bailey have no conflicts of interest to declare. Gerry Dawson and Colin Dourish are Directors, employees and shareholders of P1vital Ltd who funded this research. David Nutt has provided consultancy services to and/or received honoraria, grants or clinical trial payments from Pfizer, GSK, Novartis, Cypress, Lilly, Janssen, Lundbeck, BMS, Astra Zeneca, Servier, Hythiam, Sepracor, Neurosearch, Reckitt Benkiser, Schering-Plough, Wyeth, Cephalon and MSD, and holds shares in P1vital Ltd. He is an advisor to the British National Formulary, the MRC, the GMC and the Department of Health; President of the European College of Neuropsychopharmacology; member of the European Brain Council; President-elect of the British Neuroscience Association; and chair of the Independent Scientific Committee on Drugs (UK). He is the editor of the *Journal of Psychopharmacology* and on the editorial board of *Psychopharmacology* and *Depression and Anxiety*.

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