

Research Article

BEHAVIORAL AND CARDIOVASCULAR EFFECTS OF 7.5% CO₂ IN HUMAN VOLUNTEERS

Jayne E. Bailey, M.Sc.,¹ Spilios V. Argyropoulos, M.R.C.Psych., Ph.D.,¹ Adrian H. Kendrick, Ph.D.,² and David J. Nutt, D.M., F.R.C.Psych.^{1*}

The study of carbon dioxide (CO₂) inhalation in psychiatry has a long and varied history, with recent interest in using inhaled CO₂ as an experimental tool to explore the neurobiology and treatment of panic disorder. As a consequence, many studies have examined the panic-like response to the gas either using the single or double breath 35% CO₂ inhalation or 5–7% CO₂ inhaled for 15–20 min, or rebreathing 5% CO₂ for a shorter time. However, this lower dose regime produces little physiological or psychological effects in normal volunteers. For this reason we have studied the effects of a higher concentration of CO₂, 7.5%, given over 20 min. Twenty healthy volunteers were recruited to a double blind, placebo-controlled study where air and 7.5% CO₂ were inhaled for 20 min. Cardiovascular measures and subjective ratings were obtained. When compared to air, inhaling 7.5% CO₂ for 20 min increases systolic blood pressure and heart rate, indicating increased autonomic arousal. It also increases ratings of anxiety and fear and other subjective symptoms associated with an anxiety state. The inhalation of 7.5% CO₂ for 20 min is safe for use in healthy volunteers and produces robust subjective and objective effects. It seems promising as an anxiety provocation test that could be beneficial in the study of the effects of anxiety on sustained performance, the discovery of novel anxiolytic agents, and the study of brain circuits and mechanisms of anxiety. Depression and Anxiety 21:18–25, 2005. © 2005 Wiley-Liss, Inc.

Key words: carbon dioxide; anxiety; autonomic activation; human model

INTRODUCTION

The study of carbon dioxide (CO₂) inhalation in psychiatry has a long and varied history [Leake, 1973]. CO₂ was recognised as an anaesthetic agent in the late nineteenth and early twentieth century and it was believed that these effects could be used to produce 'a quietness of the central nervous system'. Further experiments to explore this idea led to some interesting findings in mentally disturbed patients. The administration of 30% CO₂/70% O₂ caused a catatonic schizophrenic to respond by sitting up and speaking [Loevenhart et al., 1929; Meduna, 1948], but did not achieve lasting remission. The rapid coma technique of CO₂ inhalation therapy (70% CO₂/30% O₂) was thought to be a useful therapy in the treatment of heroin addiction [LaVerne, 1953]. It was also proposed that CO₂ inhalation might be used to treat maladaptive anxiety responses, such as free-floating anxiety, specific

anxiety syndromes, and panic attacks [Wolpe, 1987]. It was in the early 1980s, however, when work being carried out on both sides of the Atlantic produced the

¹Psychopharmacology Unit, University of Bristol, The Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Dorothy Hodgkin Building, Bristol, United Kingdom

²Department of Respiratory Medicine, Bristol Royal Infirmary, Bristol, United Kingdom

*Correspondence to: D.J. Nutt, Psychopharmacology Unit, University of Bristol, The Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Dorothy Hodgkin Building, Whitson Street, Bristol BS1 3NY, United Kingdom.
E-mail: David.J.Nutt@bristol.ac.uk

Received for publication 10 May 2004; Revised 1 December 2004; Accepted 8 December 2004

DOI 10.1002/da.20048

Published online 21 March 2005 in Wiley InterScience (www.interscience.wiley.com).

current interest in CO₂ as an experimental tool, particularly to explore the neurobiology and treatment of panic disorder [Gorman et al., 1984; van den Hout and Griez, 1984].

Since this time, interest in CO₂ as a model of experimental anxiety has focussed on the inhalation of low concentrations (5–7% CO₂) over 15–20 min [Gorman et al., 1988], or a high concentration (35%) delivered via a single vital capacity inhalation [Verburg et al., 2001]. Both models are well validated and documented and seem to be reliable in producing panic symptoms in panic disorder patients, but it is generally thought that healthy normal volunteers do not react as strongly. The majority of published studies have used volunteer groups as a control for panic disorder patient groups and evaluation criteria have often been panic-specific, rather than a general examination of the anxiety and arousal responses evoked.

We have examined the effects of a single inhalation of 35% CO₂ in normal volunteers [Argyropoulos et al., 2002] and have explored the response to CO₂ as a stress stimulus rather than as an anxiety-panic specific response. We reported a robust physiological response comprising activation of the autonomic nervous system and the hypothalamo-pituitary-adrenal (HPA) axis, plus increases in subjective fear that did not amount to panic. These findings suggest that the 35% CO₂ inhalation is indeed producing a stress response, shown by activation of the HPA axis in normal volunteers. In addition, this has been replicated recently by van Duinen et al. [2005] and by Kaye et al. [2004] who also report increases in ACTH, prolactin and noradrenaline in response to inhalation of 35% CO₂ in volunteers.

When considering anxiety provocation, most human models are panic-specific, in that the anxiety produced tends to be acute and short-lived. In addition, not all anxiety provocation models produce symptoms in healthy volunteers, but may only produce symptoms in patients with an anxiety disorder [Nutt, 2001]. It would, therefore, be of use to model a more chronic anxiety state so that additional ratings or measures of performance can be studied.

To explore such a model, we have examined the effects of inhaling 7.5% CO₂ for 20 min in normal healthy volunteers. The anxiety state produced by this concentration of CO₂ seems to be greater than that produced by 5% [Stegen et al., 1998; Woods et al., 1988] and in our experience, a different state is produced to that of the 35% CO₂ single inhalation [Argyropoulos et al., 2002]. This study outlines our research findings and describes the psychological and physiological effects of inhaling 7.5% CO₂ in healthy human participants.

SUBJECTS AND METHODS

ETHICAL CONSIDERATIONS

The study protocols were approved by the local research ethics committee. All subjects gave written

informed consent before their participation. The subject information sheets provided details on the expected effects of inhaling CO₂ and it was explained that individuals exhibit different sensitivities to the stimulus, which may affect outcome.

VOLUNTEERS

Twenty volunteers (6 women, 13 men) with a mean age of 25.2 years (range = 19–40) were recruited from within the hospital and university campus. Before inclusion, all subjects were given a physical examination, including ECG, to ensure good medical health and were interviewed by a psychiatrist to ensure no history of, or first-degree relatives with, anxiety or panic disorder. Other exclusion criteria were: current or history of drug or alcohol abuse/dependence, smoking >5 cigarettes/day, current or history of cardiovascular, respiratory or renal disease, hypertension, migraine, 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 before testing. A pregnancy test was carried out on female subjects before entry. Subjects were paid for their involvement.

STUDY DESIGN

This was a placebo-controlled (air) study, with 14 of 20 subjects receiving air first followed by the 7.5% CO₂ challenge. The interval between the two gas deliveries was 15 min.

PROCEDURE

Subjects reported to the Psychopharmacology Unit clinical research facility. Alcohol was avoided for 36 hr and caffeine for 12 hr before testing and subjects were instructed to eat a light meal at least 1 hr before attending the research unit. Before testing, questions were asked to determine that the subject was still fit to undergo the study procedure and that health status had not changed since the screening day. They were then settled into a comfortable chair in the respiratory testing room for the test period, subjects were put at their ease and baseline measures were commenced after a settling period of approximately 15 min.

DELIVERY OF GAS

Gas mixture was CO₂ 7.5%/O₂ 21%/N 71.5% or piped air and this was delivered via a nasal-oral exercise face mask (Hans Rudolf, Kansas), which was attached to a 500-L bag. Breathing pattern was monitored during each 20-min inhalation period using a pneumotachograph/integrator system (in-house) and recorded onto a multi-channel chart recorder (Lectromed, Herts, UK). The actual breath-by-breath changes in % CO₂ and % O₂ were monitored throughout using a respiratory mass spectrometer (QP9000; PK Morgan, Kent, UK) and recorded simultaneously onto the chart recorder.

At least two investigators remained with the subject throughout the procedure.

CARDIOVASCULAR MEASURES

Beat-to-beat blood pressure and heart rate measures were obtained using the Finapres (Ohmeda, Englewood, CO). The subject wore a finger cuff with a photosensitive cell connected via a servo-controlled pump, which inflates the cuff to maintain a constant pressure in the finger [see Coupland et al., 1995 for further details]. A constant recording was made during the inhalation of each gas mixture. During this time the subject's hand rested on the arm of the chair to minimise movement.

SUBJECTIVE RATINGS

Visual analogue rating scales (VAS) were used, measured on 100 mm line, anchored from 0 ("not at all") to 100 ("the most...ever"). The individual items were labeled as: alert, anxious, fearful, relaxed, happy, feel like leaving the room, feel paralysed, tense, irritable, nervous, worried. These scales provide a good estimate of rapid changes of aspects of mood states [Bond and Lader, 1974]. The individual items were chosen because of their relevance to anxiety or stress responses. Assessments were made at baseline, at the end of inhalation (+20 min) and 10 min after the end of the inhalation, just before the second inhalation (+30 min). Immediately after each inhalation subjects were asked to rate how they felt at the peak effects of the gas, this rating being "peak".

The panic symptom inventory (PSI) lists 34 symptoms related to panic anxiety and the associated autonomic arousal, with the option of rating 0 = not at all, 1 = slight, 2 = moderate, 3 = severe, or 4 = very severe. It has been used in studies of panic provocation [Bell et al., 2002; Nutt et al., 1990] and our previous 35% CO₂ studies [Argyropoulos et al., 2002]. The PSI was adapted from Clark and Hemsley [1982] and was

administered at baseline for peak effects of inhalation and at the end of each inhalation.

The Spielberger State Anxiety Inventory (SSAI) [Spielberger, 1983] was used to measure state anxiety at baseline and 10 min after the end of each inhalation period.

STATISTICS

Comparisons between air and CO₂ were made using repeated measures ANOVA for physiological data with gas as within subject effect. Subjective VAS and PSI data were analysed using Wilcoxon's signed rank test and a Student's paired *t*-test was carried out on the SSAI data. Software used for the analysis was SPSS version 10.1 for Windows. Because the delivery of gas was not balanced, we considered that too few subjects received CO₂ first to study order effects.

RESULTS

CARDIOVASCULAR

The 7.5% CO₂ inhalation increased heart rate and blood pressure early in the 20-min period (Fig. 1). Blood pressure and heart rate values recorded during the inhalation period were expressed as a mean for the total duration. During CO₂ inhalation, systolic blood pressure (SBP) and heart rate (HR) were significantly increased compared to during air inhalation. Diastolic blood pressure (DBP) was not significant. Data are shown in Table 1. A typical cardiovascular response to 7.5% CO₂ is shown in Figure 1. Of note, the values during air inhalation suggest raised BP. This may be due to the experimental situation or may be due to the nature of data collection because in this experiment Finapres was used primarily to measure change rather than absolute values.

VISUAL ANALOGUE SCALES

Data are expressed as a change score from the -10 baseline value. There are significant effects of gas at peak and at +20 min (i.e., end of inhalation). For peak effects of CO₂, 10 of 11 ratings are significantly different from air (Table 2). The subjective reporting of

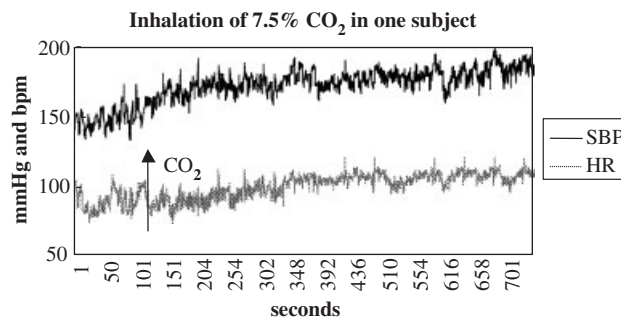


Fig. 1. Continuous measurement of blood pressure and heart rate during inhalation of 7.5% CO₂ for one subject, demonstrating increases in response to CO₂. SBP, systolic blood pressure; HR, heart rate. Arrow indicates time CO₂ started.

TABLE 1. Heart rate and blood pressure during 20 min inhalation of air or 7.5% CO₂

Value	Air*	CO ₂ *	ANOVA		
			<i>P</i>	<i>df</i>	<i>F</i>
SBP	154 (17.7)	172 (16.7)	<0.001	1,19	19.5
DBP	91 (18.2)	94 (14.8)	ns		
HR	76 (9.3)	84 (14.7)	<0.001	1,19	19.3

*Values are expressed as mean (*sd*).

N = 20.

ns, not significant.

‘alertness’ was not significantly different between the gases at this, or any other, time point.

At +20 min after start of inhalation, 7 of 11 ratings are significantly different for air and CO₂ (Table 3).

TABLE 2. VAS data, change from baseline values for peak effects of gas

Rating	Peak air	Peak CO ₂
Anxious	2 (2.1)	25 (5.8) ^b
Fear	1 (2.9)	24 (4.5) ^b
Feel like leaving	3 (2.5)	23 (5.8) ^b
Happy	-10 (2.9)	-27 (4.0) ^c
Irritable ^a	2 (2.1)	16 (6.9) ^d
Nervous ^a	5 (2.3)	17 (3.4) ^c
Paralysed	4 (2.6)	15 (4.2) ^c
Relaxed	-13 (5.5)	-35 (4.9) ^b
Tense	4 (2.9)	29 (5.1) ^b
Worried ^a	1 (1.6)	18 (4.7) ^c

Values are expressed as mean (sem); n = 20.

^an = 12.

Wilcoxon signed rank test: ^bP = .001; ^cP = .01; ^dP < .05.

TABLE 3. VAS data, change from baseline values for +20 min of gas

Rating	Peak air	Peak CO ₂
Anxious	-2 (2.4)	7 (2.8) ^c
Fear	8.7 (3.2)	3.5 (6.3)
Feel like leaving	1 (1.9)	12 (4.5) ^b
Happy	-5 (4.5)	-16 (2.8) ^c
Irritable ^a	-1 (2.3)	4.6 (3.0) ^d
Nervous ^a	-1.7 (2.5)	2.1 (2.4)
Paralysed	2 (1.1)	7 (1.9) ^d
Relaxed	1 (5.7)	-29 (4.9) ^b
Tense	-2 (3.5)	16 (5.7) ^c
Worried ^a	-2.1 (1.6)	0.8 (2.6)

Values are expressed as mean (sem); n = 20.

^an = 12.

Wilcoxon Signed rank test: ^bP = .001; ^cP = .01; ^dP < .05.

There were no significant changes from baseline at the +30 minute time point.

PANIC SYMPTOM INVENTORY

The PSI total score was significantly different at peak effects of CO₂ compared with air (air = 8.9 [±1.9]; CO₂ = 26.5 [±3.4], Wilcoxon P < .001) and at +20 min (air = 5.4 [±5.8]; CO₂ = 13.1 [±7.8], Wilcoxon P = .001). Total score for each subject at baseline, peak air, and peak CO₂ are shown in Figure 2. Subjects 12, 17, and 18 scored highly in response to CO₂.

SPIELBERGER STATE ANXIETY INVENTORY

There were significant differences between baseline and post-CO₂ inhalation (P = .009) and post-air compared to post-CO₂ (P = .002). No difference between baseline and post-air were seen. Data are shown in Figure 3.

RESPIRATORY MEASURES

Equipment failure prevented collection of respiratory variables for all but five subjects. Figure 4 shows end tidal CO₂ measures for five subjects during inhalation of 7.5% CO₂ (top section) and air (lower section), with air showing more inter-individual variability.

DISCUSSION

PHYSIOLOGICAL

This experiment demonstrates measurable physiological effects of inhaling 7.5% CO₂ in normal healthy, non-anxious volunteers. These effects seem to be of fairly rapid onset, as demonstrated by the rise in blood pressure and heart rate shown in Figure 1, and are sustained for the duration of the inhalation period.

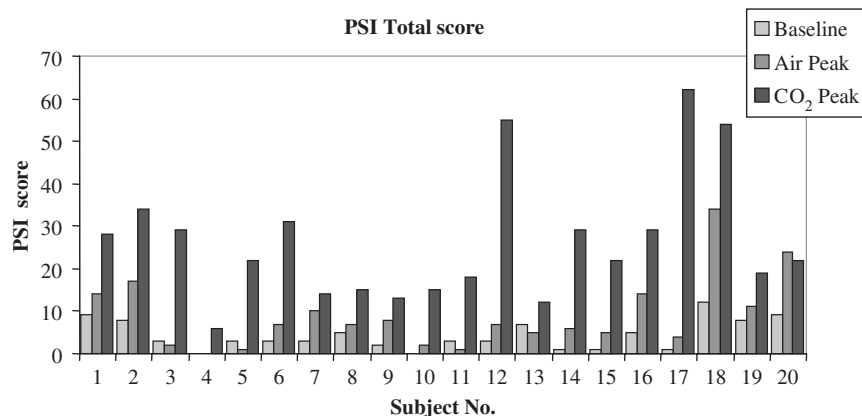


Fig. 2. PSI total score for baseline, peak response of air and CO₂ inhalation for each subject undergoing the procedure.

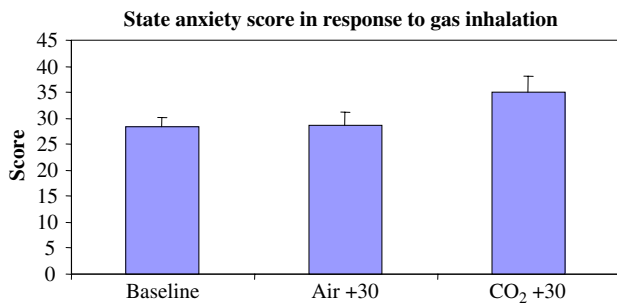


Fig. 3. Mean total score for Spielberger State Anxiety Inventory for baseline, air +30 min and CO₂ +30 min. Significant differences between baseline and post-CO₂ inhalation ($P = .009$) and post air compared to post-CO₂ ($P = .002$).

This cardiovascular response to 7.5% CO₂ differs to that produced by 35% CO₂ where blood pressure is increased but a bradycardia is also seen [Argyropoulos et al., 2002].

Although there is a large published literature on the physiological and psychological effects of the 35% CO₂ single inhalation and the 5% CO₂ inhalation for 15–20 min, there are fewer reports of the effects of 7–10% CO₂. One of the most interesting reports is from the early 1960s [Sechzer et al., 1960] when the authors demonstrated that markers of autonomic arousal were increased when male volunteers inhaled 7–14% CO₂ for periods of 10–20 min. They reported increases in respiratory minute volume, blood pressure, and heart rate. In addition, plasma levels of epinephrine, norepinephrine, and 17-OH corticosteroids were increased, suggesting sympathoadrenal arousal during CO₂ inhalation.

Almost three decades on from Sechzer's study, Woods et al. [1988] examined the biochemical and

physiologic effects of inhaling 5% and 7.5% CO₂ in healthy subjects. They reported a dose-related increase in blood pressure, heart rate, and respiratory rate in response to inhaling the gas for 15 min. There was a non-significant increase in plasma cortisol and prolactin after inhaling 7.5% CO₂, but no effect of the gas on the noradrenaline metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), or growth hormone (GH). Of interest, like Sechzer et al. [1960], this group also reported that inhalation of 7.5% CO₂ caused headache in many of the volunteers, an observation we also made, but did not formally rate. In approximately 60% of our subjects headache after CO₂ was reported and we have since made a history of frequent headache or migraine an exclusion criteria for our studies.

In Rhesus monkeys using CO₂ concentrations of 5%, 7.5%, and 10%, inhaled for 180 min, Krystal et al. [1989] reported dose-dependent increases in respiratory rate, MHPG, GH and cortisol (significant for 7.5% and 10% CO₂), and prolactin (significant at 10% CO₂). A general behavioral activation was observed within the first 15 min of inhalation, quantified by an increase in head and body turns. These results suggested a general autonomic arousal and a hormonal stress reaction in response to CO₂ in this species.

Much of the interest in CO₂ as a respiratory challenge stems from Klein's theory in which he suggests that panic disorder is due to an increased sensitivity of the central chemoreceptors to carbon dioxide. This means that these patients are more likely to trigger these neurons that leads to a false 'suffocation alarm', that in turn leads to panic [Klein, 1993]. This theory has been tested with carbon dioxide challenges to examine and compare ventilatory response and respiratory variables in patient populations and volunteers by a number of groups [Gorman et al., 1988; Lousberg et al., 1988; Pain et al., 1988; Papp

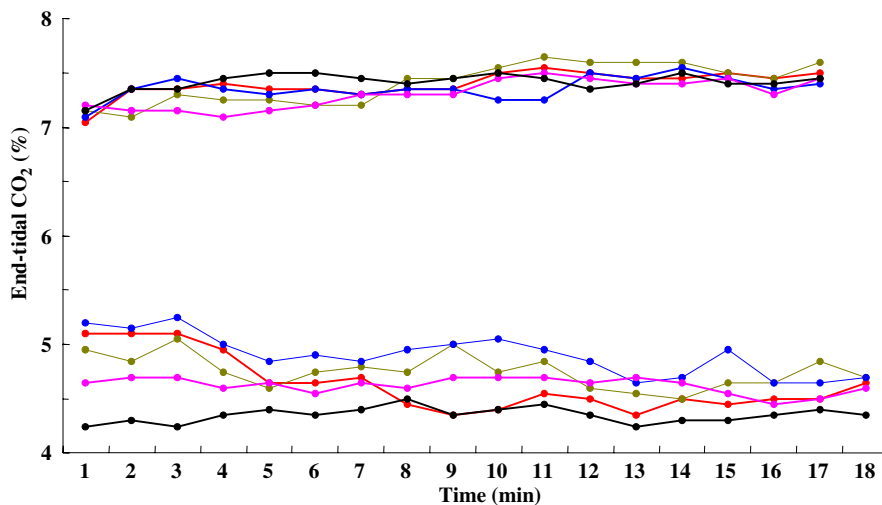


Fig. 4. End tidal CO₂ measures for five subjects during inhalation of 7.5% CO₂ (top section) and air (lower section), with air showing more inter-individual variability. [Color figure is available in the online issue, at www.interscience.wiley.com.]

et al., 1995, 1997]. These studies have yielded different and inconclusive results. More recently, however, Gorman et al. [2001] felt it important to conduct a further study with an independent cohort of patients to assess whether increased ventilatory response is patient-specific (i.e., are only patients with panic disorder sensitive or is any person who panics, regardless of diagnosis, more sensitive?).

Patients with a diagnosis of panic disorder, major depression, pre-menstrual dysphoric disorder, and normal volunteers underwent the 5% and 7% CO₂ challenge. Continuous respiratory measures of tidal volume, respiratory rate, minute volume, and end tidal CO₂ were conducted. It would be interesting to see whether similar blood pressure and heart rate findings were observed across patient groups. Unfortunately, cardiovascular parameters were not reported. The authors concluded that having a panic attack in response to CO₂ was more important than having a diagnosis of panic disorder in distinguishing response and that rather than, or in addition to, abnormal chemoreceptors, central brain circuits are implicated. They propose a theory that 'panic' to CO₂ involves a more generalised fear response implicating the amygdala and neural 'fear' circuit. The findings from Argyropoulos et al. [2002] showing increased fear and an HPA axis activation after 35% CO₂ inhalation would support this theory.

PSYCHOLOGICAL

It is clear that in this study 7.5% CO₂ increased subjective fear and anxiety in these healthy participants. The subjective changes in response to 7.5% CO₂ reported using the VAS show a heightened 'threat' situation, with ratings of anxious, fear, feel like leaving the room, and tense all increasing in response to 7.5% CO₂ and ratings of relaxed and happy significantly decreasing. These changes were apparent at peak effects of gas during the 20-min inhalation period and also at the end of the inhalation (20-min time-point) as shown in Tables 2 and 3. A limitation of these time-points, however, is that "peak" was a retrospective rating. Perhaps it would have been of greater interest to rate at 5-min intervals during the inhalation or even continuously.

The total score on the PSI was significantly higher for 7.5% CO₂ compared to air. Interestingly the total PSI score for peak effects of 7.5% CO₂ is similar to that found in our previous study with 35% CO₂ (26.5 versus 21) [Argyropoulos et al., 2002] suggesting a similar state of arousal. There are many similarities between the symptoms produced by the two methods of gas inhalation, but there are also differences, with a greater percentage of subjects reporting increased sweating, tremor, tension, and tight muscles on 7.5% CO₂. Three subjects scored highly on the PSI in response to 7.5% CO₂. These individuals demonstrated a marked sensitivity to the gas, but recovered

rapidly with no sequelae. Individual variability of response is shown in Figure 2.

The data from the Spielberger state anxiety inventory suggests that a residual anxiety is still present for up to 10 min after completion of the CO₂ inhalation, as indicated by the significant increase in the score (Fig. 3). This is not reflected in the VAS scores, however, and it must be noted that the mean Spielberger score is still within the range for normal volunteers, with anxious patients reporting a mean score of 49 [Spielberger, 1983].

Interestingly, returning to the Sechzer [1960] study, no formal ratings of subjective state were measured, but descriptions of the procedure being "horrible", "unbearable", "like strangling", or "suffocating" were reported. Other subjects in the Sechzer study reported a fear of calamity or feeling of impending death, and profuse sweating and headache were not uncommon. These reports are interesting and clearly describe a fairly intense state of anxiety, perhaps even a panic attack. The subjective reports from the Woods et al. [1988] study showed a dose response of CO₂, with significantly greater ratings of fear for 7.5% CO₂ (VAS change = 30 of 100) compared to 5% CO₂ (VAS change = 3 of 100).

Many of the published studies using CO₂ to provoke panic anxiety in patients with panic disorder and other anxiety disorders, have used primarily panic-specific criteria (i.e., using the 12 or 13 items from DSM-III-R or DSM-IV [American Psychiatric Association, 1987 and 1994] to determine the panic response to the challenge) rather than break down specific symptomatology. This response is often expressed as a total score and because many different scales are in use, it is not always easy to interpret findings, particularly in normal volunteers. We did not formally rate whether the subjects in our studies experienced a panic attack because we were not testing this hypothesis. Many published articles on CO₂ provocation have discussed whether a panic response is a true panic attack, and Sanderson and Wetzler [1990] have reviewed the difference in defining laboratory panic and the consequent differences in reported rates of CO₂-induced panic. At this time, they suggested specific criteria should be applied to determine whether or not a panic attack occurred [Sanderson et al., 1989], but today, there are still different definitions in use. For example, van Beek and Griez [2000] determine a CO₂-induced panic attack by an increase of anxiety of ≥ 25 on the visual analogue scale (0–100), combined with an increase in panic symptomatology. If we apply this criterion to the subjects who participated in our study, 30% (6 of 20) would be classified as experiencing a panic attack. No subject asked to terminate the procedure, however, and on no occasion did the subjects seem unduly distressed by the experience.

In our study, each volunteer was given an information sheet detailing the possible CO₂-induced symptoms. They were also told that individuals may be

differently sensitive and that it was difficult to know in advance how they may respond. Whether instructional conditions could change outcome to 5 and 7% CO₂ inhalation was examined by Welkowitz et al. [1999]. Panic disorder patients and control subjects were assigned to one of three groups; reassurance (reiterated safety instructions), control dial (were able to reduce CO₂ concentration), and basic instructions (standard information). All subjects underwent the same procedure regardless of instructional set. The results suggest that such manipulation does not affect panic rate in patients and no significant differences were found for any of the biological variables. It seems that response to CO₂ is a robust biological effect and is independent of cognitive manipulation

CONCLUSIONS

In developing a model for the study of human anxiety, it is important to be able to evaluate the effects of the challenge and thus it is imperative to select a reliable tool to measure the subjective response. Our data shows that a range of responses on a questionnaire and a selection of visual analogue scales gives a good overview of subjective changes that occur in response to inhalation of 7.5% CO₂ and also demonstrates a reasonable recovery 10 min after the gas inhalations. In addition, a response to a challenge should monitor physiological changes as well as subjective reports. This important issue is discussed further by Wilhelm and Roth [2001], who argue that physiological anxiety symptoms (such as palpitations, sweating, chest pain), could be evaluated within the laboratory and that the findings of such monitoring may well lead to a refined assessment of anxiety disorders. Wilhelm and Roth [2001] also remind us that pharmaceutical companies promote the biological basis of anxiety disorders, yet the outcome of any treatment is always evaluated by self-report.

To further this research it will be of interest to show an attenuated response to anxiolytic medication. Preliminary data from our studies suggest that the prior administration of the benzodiazepine lorazepam does reduce some of the symptoms produced by 7.5% CO₂ [Nutt and Bailey, 2002, 2004]. Further investigation of the effects of established GAD treatments, such as paroxetine and venlafaxine, on subjective and physiological responses to 7.5% CO₂ is required. Findings from this and our previous studies have produced some strong correlations between subjective and physiological responses to CO₂. This has led us to postulate that a common mechanism may mediate these responses and have proposed that the noradrenergic system may be the key mediator [Bailey et al., 2003]. With this in place, it is of interest to test this theory with further pharmacological probes and neurochemical measures. In addition, it would be of interest to observe how patients with an anxiety disorder would respond to the 7.5% CO₂ challenge.

Hoehn-Saric and McLeod [2000] suggest that patients with chronic anxiety disorders show a weaker physiological response to laboratory stressors than do normal control subjects. They explain this as a 'diminished physiological flexibility' and it would be of interest to repeat our experiments examining the psychophysiological response to 7.5% CO₂ in patients with GAD or PD. It is of course also important to elucidate whether the responses reported here are reliably repeatable over time or whether they are attenuated after repeated exposure.

REFERENCES

- American Psychiatric Association. 1987. Diagnostic and Statistical Manual of Mental Disorders. Third edition, revised, DSM-III-R. Washington, D.C.: APA.
- American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders. Fourth edition, DSM-IV. Washington, D.C.: APA.
- Argyropoulos SV, Bailey JE, Hood SD, Kendrick AH, Rich AS, Laszlo G, Nash JR, Lightman S, Nutt DJ. 2002. Inhalation of 35% CO₂ results in activation of the HPA axis in healthy volunteers. *Psychoneuroendocrinology* 27:715–729.
- Bailey JE, Argyropoulos SV, Lightman SL, Nutt DJ. 2003. Does the brain noradrenaline network mediate the effects of the CO₂ challenge? *J Psychopharmacol* 17:252–259.
- Bell C, Forshall S, Adrover M, Nash J, Hood S, Argyropoulos S, Rich A, Nutt DJ. 2002. Does 5-HT restrain panic? A tryptophan depletion study in panic disorder patients recovered on paroxetine. *J Psychopharmacol* 16:5–14.
- Bond AJ, Lader MH. 1974. The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47:211–218.
- Clark DM, Hemsley DR. 1982. The effects of hyperventilation; individual variability and its relation to personality. *J Behav Ther Exp Psychiatry* 13:41–47.
- Coupland NJ, Bailey JE, Wilson SJ, Horvath R, Nutt DJ. 1995. The effects of clonidine on cardiovascular responses to standing in healthy volunteers. *Clin Auton Res* 5:171–177.
- Gorman JM, Askanazi J, Liebowitz MR, Fyer AJ, Stein J, Kinney JM, Klein DE. 1984. Response to hyperventilation in a group of patients with panic disorder. *Am J Psychiatry* 141:857–861.
- Gorman JM, Fyer MR, Goetz R, Askanazi J, Liebowitz MR, Fyer AJ, Kinney J, Klein DE. 1988. Ventilatory physiology of patients with panic disorder. *Arch Gen Psychiatry* 45:31–39.
- Gorman JM, Kent J, Martinez J, Browne S, Coplan J, Papp LA. 2001. Physiological changes during carbon dioxide inhalation in patients with panic disorder, major depression and premenstrual dysphoric disorder. *Arch Gen Psychiatry* 58:125–131.
- Hoehn-Saric R, McLeod DR. 2000. Anxiety and arousal: Physiological changes and their perception. *J Affect Disord* 61:217–224.
- Kaye J, Buchanan F, Kendrick A, Johnson P, Lowry C, Bailey J, Nutt DJ and Lightman SL. 2004. Acute carbon dioxide exposure in healthy adults: Evaluation of a novel means of investigating the stress response. *J Neuroendocrinol* 16:256–264.
- Klein DE. 1993. False suffocation alarms, spontaneous panics and related conditions: An integrative hypothesis. *Arch Gen Psychiatry* 50:306–317.
- Krystal JH, Woods SW, Levesque M, Heninger C, Heninger GR. 1989. The effects of carbon dioxide inhalation on plasma MHPG, plasma hormones, respiratory rate and behavior in the rhesus monkey. *Life Sci* 45:1657–1663.

- LaVerne AA. 1953. Rapid coma technique of carbon dioxide inhalation therapy. *Dis Nerv Syst* 14:141–144.
- Leake CD. 1973. Prologue and epilogue of carbon dioxide therapy: Carbon dioxide as a physiological agent. *Behav Neuropsychiatry* 5:10–12.
- Lovenhart AS, Lorenz WF, Waters M. 1929. Cerebral stimulation. *JAMA* 92:11.
- Lousberg H, Griez E, van den Hout MA. 1988. Carbon dioxide chemosensitivity in panic disorder. *Acta Psychiatr Scand* 77: 214–218.
- Meduna LJ. 1948. Alterations of neurotic patterns by use of carbon dioxide inhalations. *J Nerv Ment Dis* 108:373.
- Nutt DJ. 2001. The pharmacology of human anxiety. In: Griez EJL, Faravelli C, Nutt D, Zohar J, editors. *Anxiety disorders—an introduction to clinical management and research*. Chichester: John Wiley and Sons. p 309–324.
- Nutt DJ, Bailey JE. 2002. The neurobiological basis of GAD. In: Nutt DJ, Rickels K, Stein DJ, editors. *Generalised anxiety disorder: Symptomatology, pathogenesis and management*. London: Martin Dunitz Limited.
- Nutt DJ, Bailey JE. 2004. Can CO₂ be used to model different anxiety disorders? *Eur Neuropsychopharmacol* 14(Suppl):S153.
- Nutt DJ, Glue P, Lawson C, Wilson S. 1990. Flumazenil provocation of panic attacks: Evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Arch Gen Psychiatry* 47:917–925.
- Pain MCF, Biddle N, Tiller JWJG. 1988. Panic disorder, the ventilatory response to carbon dioxide and respiratory variables. *Psychosom Med* 50:541–548.
- Papp LA, Martinez JM, Klein DE, Coplan JD, Gorman JM. 1995. Rebreathing tests in panic disorder. *Biol Psychiatry* 38:240–245.
- Papp LA, Martinez JM, Klein DE, Coplan JD, Norman RG, Cole R, de Jesus MJ, Ross D, Goetz R, Gorman JM. 1997. Respiratory psychophysiology of panic disorder: Three respiratory challenges in 98 subjects. *Am J Psychiatry* 154:1557–1565.
- Sanderson WC, Rapee RM, Barlow DH. 1989. The influence of an illusion of control on panic attacks induced via inhalation of 5.5% carbon dioxide-enriched air. *Arch Gen Psychiatry* 46:157–162.
- Sanderson WC, Wetzler S. 1990. Five percent carbon dioxide challenge: Valid analogue and marker of panic disorder? *Biol Psychiatry* 27:689–701.
- Sechzer PH, Egbert LD, Linde HW, Cooper DY, Dripps RD, Price HL. 1960. Effect of CO₂ inhalation on arterial pressure, ECG and plasma catecholamines and 17-OH corticosteroids in normal man. *J Appl Physiol* 15:454–458.
- Spielberger CD. 1983. *Manual for the State-Trait Anxiety Inventory (Form Y)*. Mountain View, CA: Consulting Psychologists Press, Inc.
- Stegen K, Neujens A, Crombez G, Hermans D, Van de Woestijne KP, Van den Bergh O. 1998. Negative affect, respiratory reactivity, and somatic complaints in a CO₂ enriched air inhalation paradigm. *Biol Psychol* 49:109–122.
- van Beek N, Griez E. 2000. Reactivity to a 35% CO₂ challenge in healthy first-degree relatives of patients with panic disorder. *Biol Psychiatry* 47:830–835.
- van Duinen MA, Schruers KRJ, Maes M, Griez EJL. 2005. CO₂ challenge results in HPA activation in healthy volunteers. *J Psychopharmacol* 19:243–247.
- van den Hout MA, Griez E. 1984. Panic symptoms after inhalation of carbon dioxide. *Br J Psychiatry* 144:503–507.
- Verburg K, Perna G, Griez EJL. 2001. A case study of the 35% CO₂ challenge. In: Griez EJL, Faravelli C, Nutt D, Zohar J, editors. *Anxiety disorders—an introduction to clinical management and research*. Chichester: John Wiley and Sons. p 341–357.
- Welkowitz LA, Papp L, Martinez J, Browne S, Gorman JM. 1999. Instructional set and physiological response to CO₂ inhalation. *Am J Psychiatry* 156:745–748.
- Wilhelm FH, Roth WT. 2001. The somatic symptom paradox in DSM-IV anxiety disorders: Suggestions for a clinical focus on psychophysiology. *Biol Psychol* 57:105–140.
- Wolpe J. 1987. Carbon dioxide inhalation treatments of neurotic anxiety: An overview. *J Nerv Ment Dis* 175:129–133.
- Woods SW, Charney DS, Goodman WK, Heninger GR. 1988. Carbon-dioxide-induced anxiety: Behavioral, physiologic, and biochemical effects of carbon dioxide in patients with panic disorders and healthy subjects. *Arch Gen Psychiatry* 45:43–52.