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REVIEW ARTICLE

The carbon dioxide challenge test in panic disorder: a systematic review of preclinical and clinical research

Julio Mario Xerfan do Amaral,[1,2,3] Pedro Tadeu Machado Spadaro,[1,2,3] Valeska Martinho Pereira,[2,3] Adriana Cardoso de Oliveira e Silva,[2,3] Antonio Egidio Nardi[2,3]

[1]*School of Medicine, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil.* [2]*Laboratory of Panic & Respiration, Institute of Psychiatry, UFRJ, Rio de Janeiro, RJ, Brazil.* [3]*National Science and Technology Institute for Translational Medicine (INCT-TM).*

This systematic review assesses the current state of clinical and preclinical research on panic disorder (PD) in which the carbon dioxide (CO_2) challenge was used as a trigger for panic attacks (PAs). A total of 95 articles published from 1984 to 2012 were selected for inclusion. Some hypotheses for PD evolved greatly due to the reproducibility of PAs in a controlled environment using the safe and noninvasive CO_2 test. The 35% CO_2 protocol was the method chosen by the majority of studies. Results of the test report specific sensitivity to hypercapnia in PD patients of the respiratory PD subtype. The CO_2 challenge helped assess the antipanic effects of medication and non-pharmaceutical approaches such as physical exercise and cognitive behavioral therapy. The test was also used in studies about the genetic component of PD, in which twins and relatives of PD patients were analyzed.

Keywords: Panic disorder; panic attacks; carbon dioxide challenge; respiratory challenge; clinical research

Introduction

Panic disorder (PD) is a highly debilitating disease, which may affect the quality of life of affected individuals as much as major depressive disorder (MDD).[1] PD has a lifetime prevalence of approximately 3.5% in the general population. It manifests itself by acute anxiety episodes known as panic attacks (PAs), which include severe somatic symptoms (tachycardia, intense sweating, hyperventilation and chills/hot flashes), and psychic suffering (anxiety, fear of losing control, fear of going crazy).

Patients with PD always begin a description of their disorder by reporting symptoms related to breathing, the heart, the digestive tract and 'nerves.'[2] Due to the somatic symptoms, 90% of patients with PD believe they have a physical condition instead of a mental disorder. Therefore, proper diagnosis and management of these patients is important for mental health professionals and general physicians alike.

A connection between the respiratory system and PD has been reported since Klein's experiments in 1964[3] and the development of the false suffocation alarm theory in 1993.[4] This led Briggs et al.[5] to define two PD subtypes: one with prominent respiratory symptoms and one with few or non-prominent respiratory symptoms. This classification has had a great impact on patient psychopathology, response to treatment and prognosis.[6]

Correspondence: Julio Mario Xerfan do Amaral, Rua Silveira Martins, 124/403, Flamengo, CEP 22221-000, Rio de Janeiro, RJ, Brazil.
E-mail: jxerfan@ufrj.br
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New hypotheses for PD have advanced enormously in the last decades due to the possibility of reproducing PAs in a safe and controlled environment. The carbon dioxide (CO_2) challenge has been regarded as a safe and noninvasive method to provoke PAs in PD patients in preclinical and clinical research settings, and has enabled investigators to: analyze sensitivity to hypercapnia in PD, assessing the validity of Klein's suffocation alarm theory; discriminate the respiratory (R-PD) and non-respiratory (nR-PD) subtypes of PD according to patient sensitivity to hypercapnia; test the sensitivity of healthy relatives of PD patients to the CO_2 challenge; verify the ability of drugs with known pharmacokinetics to prevent, eliminate, or reduce CO_2 sensitivity in R-PDs, shedding light on the neural mechanisms of PAs and potential treatment options for PD; assess the efficacy of non-drug treatments, such as physical exercise, in preventing PAs caused by hypercapnia; and develop genetic screening for CO_2-sensitive PD.

This review sought to describe the studies performed with the CO_2 challenge as a trigger of PAs, improvements in test protocols, and the possibilities created by a method that allows reproducible and controlled investigation of an important psychiatric disorder.

Methods

This review was restricted to articles published in English. Three search queries were made using the terms panic AND carbon dioxide; panic AND CO_2; panic AND respiratory challenge in the following databases: the Institute for Scientific Information Web of Knowledge

(www.isiknowledge.com), which yielded 379 articles; and PubMed, which yielded 247 entries. After exclusion of duplicated articles and reviews and studies that did not report the specific use of the CO_2 challenge in the setting of PD, the list was narrowed to 70 original articles. Twenty-five articles from our Laboratory's archives, which did not appear in the database search, were added, for a total of 95 articles included in this review. A summary of the article selection flow is given in Figure 1.

LABPR = Laboratory of Panic & Respiration.

The reviewed articles date from 1984 to May 2012 and were divided into three groups: 1) studies of the CO_2 challenge in PD patients without the use of any medication and/or procedure aiming to control or minimize PAs, totaling 40 articles (42.10%); 2) studies of psychopharmacological interventions designed to minimize the anxiogenic effects of the CO_2 challenge in PD patients, totaling 19 articles (20%); and 3) studies that did not belong to the previous classifications, including: exposure of - exclusively - healthy subjects and relatives of PD patients to the CO_2 challenge; twin studies; and studies of non-pharmaceutical procedures aiming to control or minimize PAs (such as physical exercise and caffeine or alcohol intake), totaling 36 articles (37.89%).

Articles in group 1 are described chronologically, those in group 2 are organized by medication type, and those in group 3 are sorted by the goal of each particular research.

Results

CO_2 challenge in PD patients: medication-free studies

Of a total of 95 articles, 40 (42.10%) analyzed the CO_2 challenge in PD patients without including any sort of medication or procedure aimed at modulating the panicogenic effects of hypercapnia. Those studies discussed, for instance, the sensitivity and specificity of the CO_2 challenge, optimal methods and procedures for the challenge, and the nature of the induced PAs, as detailed below.

The 1980s

In 1983, Griez et al.[7] described anxiety-like reactions to a single inhalation of 35% CO_2. The observation of sympathomimetic effects led to a study published in the following year in which administration of propanolol (a

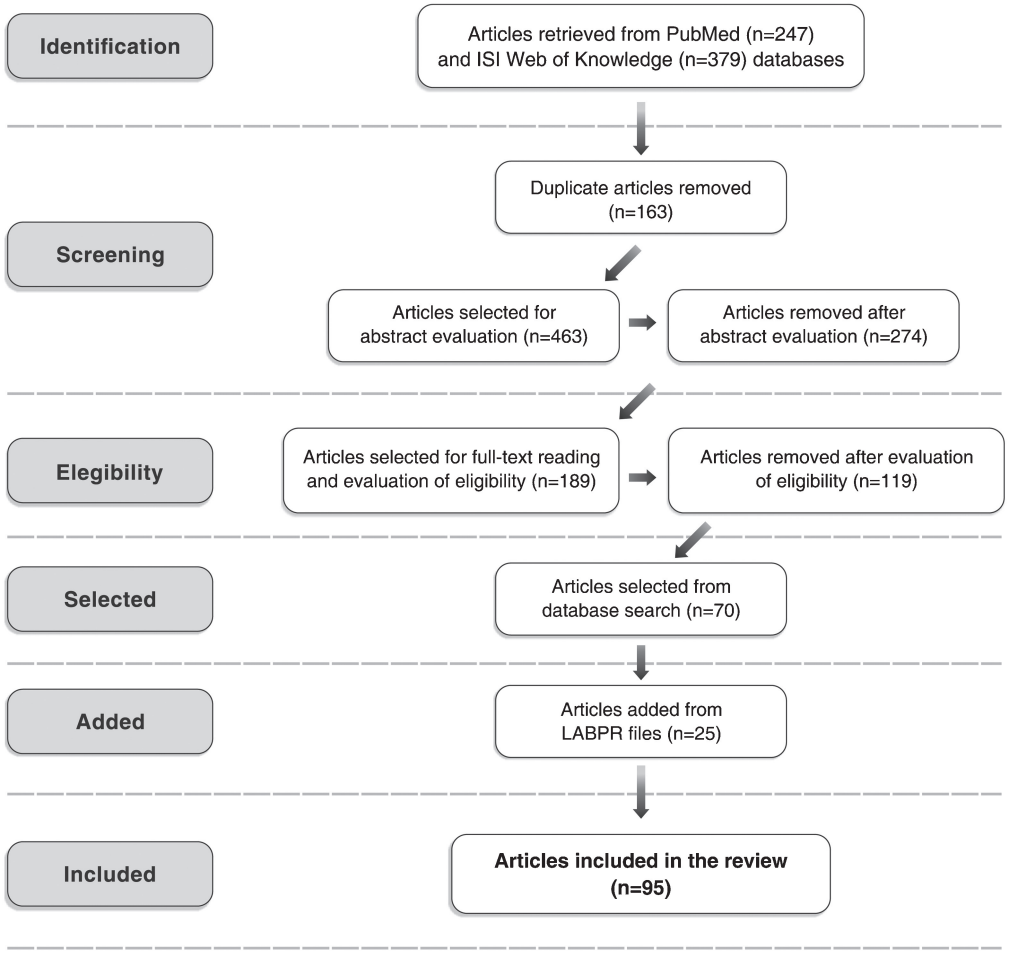


Figure 1 Article selection process

beta-blocker) diminished the reported panic symptoms induced by hypercapnia.

During the 1980s, four articles reported non-medicated tests of PAs provoked by CO_2 in PD patients. In 1987, Fryer et al.[8] subjected eight PD patients and five controls to a 35% CO_2 challenge, and found that five of the PD patients experienced PAs, whereas none of the controls did. That led to the conclusion that the 35% CO_2 challenge might be a safe and simple method to provoke PAs and a potential laboratory model for the study of PD. In the same year, Griez et al.[9] exposed 12 PD patients to the 35% CO_2 challenge and compared their response to that of 11 control subjects. Confirming the panicogenic effects of the test, the authors noted that the provoked PAs were reported by PD patients to be very similar to spontaneous PAs, and pointed to a biological susceptibility of PD patients to the concentration of CO_2 in a breathed air mixture. The precise mechanisms of this biological trait were not described at the time.

The role of chemosensitivity to CO_2 in PD was discussed by Lousberg et al.[10] in 1988, in a study that subjected 19 PD patients and 14 controls to the 7% CO_2 protocol. The significantly higher response of PD patients to the test allowed the authors to ratify the theoretical model of highly sensitive chemoreceptors in the activation of panic-like symptoms.

In 1989, Sanderson et al.,[11] using the 5.5% CO_2 test, reported that patients who declared themselves able to control the amount of inhaled CO_2 were less prone to PAs than the group that made no such statement, leading to the conclusion that psychological factors could influence response to the CO_2 test.

The 1990s

The validity of the CO_2 challenge as a biological marker for PD was confirmed by 18 out of 20 studies in this period. The first exception was published by Gorman et al.[12] in 1990, using the 35% CO_2 protocol; the authors concluded that the test would arouse a strong emotional response even in people without some sort of anxiety disorder. However, in 1993, the same author, alongside Papp et al.,[13] observed that the CO_2 challenge was significantly more able than mere respiratory stress (provoked by an increase in respiratory airway resistance) to provoke PAs in patients with both PD and social phobia. They also concluded that PD patients were much more sensitive to hypercapnia than patients with only social phobia or healthy controls.

The second exception was a study by Zandbergen et al.,[14] who, using the Read rebreathing protocol, found no difference in respiratory response between PD patients, patients with obsessive compulsive disorder (OCD), and controls. Nevertheless, using the 35% CO_2 protocol, the team of Griez et al. (including Zandbergen)[15] found that PD patients were more vulnerable to the test than OCD patients and controls, therefore acknowledging the validity of the procedure.

Zandbergen et al.[16] also compared the respiratory symptoms induced by 35% CO_2 and those induced by hyperventilation in PD patients, concluding that only the former was able to provoke significant anxiety in the subjects. The same comparison with hyperventilation, with similar results, was made by Gorman et al.,[17] this time using the 5 and 7% CO_2 protocols - noting that the 7% CO_2 was a more efficient marker.

Various comparisons of responsiveness to the CO_2 challenge between PD and other psychiatric conditions were published during this decade: Perna et al. compared patients with PD and patients with mood disorder[18] and patients with PD vs. people with occasional PAs[19]; and Verburg et al. compared PD patients with general anxiety disorder patients[20] and patients with PD alone vs. patients whose PD was associated with MDD.[21] Generally, these reports concluded that susceptibility to the CO_2 challenge is a specificity of PD, and that comorbidities did not increase responsiveness to the test.

The importance of a subjective anxiety scale in discrimination of PD and controls, instead of respiratory symptoms, was noted by Battaglia et al.[22] in a study of 147 subjects.

Schmidt et al.[23] concluded that, contrary to Klein's suffocation alarm theory, increased respiratory frequency in response to hypercapnia was a poor discriminator of PD patients and controls. Schmidt also observed the importance of cardio respiratory symptoms (over symptoms like numbness or nausea) in response to 35% CO_2.[24]

Welkowitz et al.[25] concluded that CO_2 -induced panic is a biological effect, independent of cognitive stimuli such as illusion of control over the test or reassurance of safety.

Other studies from that period by Sasaki et al.,[26] Perna et al.,[27] Bocola et al.,[28] Sanderson et al.,[29] Verburg et al.,[30] and Biber et al.[31] essentially confirmed CO_2 sensitivity as a valid marker of PD.

From 2000 to 2012

Sixteen articles from this period were analyzed, six of which discussed the different response to the CO_2 challenge between the PD subtypes (R-PD vs. nR-PD): Nardi et al.[32-36] and Papp et al.[37] These articles were unanimous in observing that, although some PD patients may experience hypercapnia-triggered PAs, those patients who show this hypercapnia might form a consistent group with similar background, manifestations, and prognosis.

This wave of studies was certainly influenced by the distinction of PD subtypes in 1993, which explained why people who had the same disorder (PD) responded differently to the panicogenic effects of hypercapnia. Among these papers, two warrant particular mention. The first, by Papp et al.,[37] subjected seven PD patients who did not panic after the CO_2 challenge to a canopy-steady state protocol (a transparent box is placed at the top of a bed, covering the subject's head), comparing their response to a control group of five people. The authors observed that anxiety levels in response to hypercapnia were hardly distinguishable between the two groups. Instead of questioning the efficacy of the CO_2 challenge to arouse PA in PD patients, this study corroborated the

existence of the nR-PD subtype. The second is the experiment conducted by Nardi et al.,[34] which compared responsiveness to the 35% CO_2 challenge between 117 PD patients (51 [43.60%] diagnosed with nR-PD and 66 [56.4%] with R-PD), with conclusive findings: R-PDs had a higher incidence of familial history of PD, a higher incidence of previous alcohol abuse, and a lower age of onset.

From this period, we selected four articles designed to compare CO_2 sensitivity between PD and other psychiatric conditions. Gorman et al.[38] and Kent et al.[39] compared sensitivity to CO_2 among patients with PD, MDD, and premenstrual dysphoric disease (PMDD). Both studies concluded that PD patients are more prone to experience a PA in response to hypercapnia, followed by PMDD patients, whereas patients with MDD seemed unresponsive to CO_2. Blechert et al.[40] compared patients with PD, patients with social phobia, and controls and found that, while PD patients did have more pronounced respiratory and autonomic deregulation in response to CO_2, similarities between the two groups (compared to controls) were found, namely in enhanced reactivity, slowed recovery, and dyspnea ratings. Comparing sensitivity to hypercapnia between patients with PD, patients with generalized anxiety disorder (GAD), and controls, Schutters et al.[41] found that patients with GAD were more responsive to CO_2 than controls, but less so than patients with PD.

In other studies from that period, Valença et al.[42] concluded that the PAs induced by the CO_2 challenge in PD patients were similar to spontaneous PAs, while Perna et al.[43] found that the Anxiety Sensitivity Index (ASI) - a measure of fear of anxiety-related bodily sensations - significantly predicted symptomatological reaction do CO_2, yet it was poorly connected to modulation of the subjective response to hypercapnia.

A trial by van Beek et al.[44] analyzed 91 PD patients and concluded that preexisting respiratory disease did not influence subject response to CO_2. Other experiments focused on respiratory physiology were conducted by Niccolai et al.,[45] who found that untreated PD patients had lower recovery rate and higher PCO_2 in response to the challenge than treated PD patients and controls; and Rassovsky et al.,[46] who found increased suffocation and respiratory responses to CO_2 in PD patients than in controls.

Finally, using electroencephalography to assess the PA provoked by CO_2 in 15 PD patients, Lopes et al.[47] were able to detect disturbances in frontal cortical processing, an imbalance of the frontal and occipital sites, common to both hemispheres, and increased right posterior activity.

Table 1 summarizes all articles reviewed in this group.

CO_2 challenge in PD patients: medication studies

Nineteen studies about the effect of drugs such as benzodiazepines, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), and beta-blockers over the panicogenic effects of the CO_2 challenge, published between the years 1991 and 2009, were included in this review.

Usually, control in these trials consisted of PD patients receiving placebo, according to each experimental design.

The articles were grouped by tested medication and are discussed below.

Beta-blockers

A report by Wheatley,[48] according to whom antagonists of β-adrenergic channels (β-blockers) could reduce physical symptoms of anxiety, motivated the first article of this section, by Van Den Hout et al.,[49] published in 1984, describing the use of propanolol to confront the anxiogenic effects of CO_2. In this trial, a 60-mg dose of propanolol was administered to 20 healthy volunteers, divided into two groups. In group 1 (n=8), a subgroup of four subjects took propanolol and breathed normal air; after 24 hours, they took placebo and breathed normal air again. Another subgroup of four subjects did the test in the opposite order (first placebo then propanolol, and breathing normal air). In group 2 (n=12), two subgroups of six patients each performed similar sequences, but instead of air, they breathed 35% CO_2. When the CO_2 challenge was preceded by intake of propanolol, reported symptoms decreased almost 20% as compared with placebo.

Benzodiazepines

This review retrieved nine articles in which PD patients medicated with benzodiazepines were subjected to the CO_2 challenge. This accounts for 37.5% of all articles in this section, and thus represents the largest group of drugs used in such investigations - which is not surprising, given the known rapid anxiolytic effects of GABA receptor enhancers. Among these, three studies used alprazolam, five used clonazepam, and one experiment compared both drugs.

The oldest article referring to alprazolam was published in 1986 by Woods et al.,[50] and demonstrated the ability of the drug to reduce the anxiogenic effects of the CO_2 challenge. A cohort of 14 PD patients and 23 healthy controls was subjected to the 5% CO_2 challenge for 15 minutes, during which time the authors described a similar ventilatory response between the two groups but a significant increase of anxiety in PD patients. Then, eight of the PD patients received 1.25 to 6.0 mg of alprazolam and the test was repeated. All the medicated subjects reported "markedly attenuated anxiety increases during rebreathing," leading the team to conclude that response to the CO_2 challenge was derived from noradrenergic or other neuronal systems instead of abnormal chemoreceptor sensitivity.

Ten years later, however, Pols et al.[51] reported little difference in anxiety levels between patients receiving an acute dose of alprazolam and those receiving placebo before the 35% CO_2 challenge. In a group of 20 PD patients, half of the subjects received 1 mg of alprazolam

Table 1 Summary of articles from group 1

Study	Main topic	CO_2 challenge test protocol
Fyer et al., 1987[8]	Sensitivity to hypercapnia	35% CO_2
Griez et al., 1987[9]	Sensitivity to hypercapnia	35% CO_2
Lousberg et al., 1988[10]	Sensitivity to hypercapnia	7% CO_2
Sanderson et al., 1989[11]	Psychological factors	5.5% CO_2
Gorman et al., 1990[12]	Sensitivity to hypercapnia	35% CO_2
Papp et al., 1993[13]	Physiological stress	35% CO_2
Zandbergen et al., 1991[14]	Sensitivity to hypercapnia	35% CO_2
Griez et al., 1990[15]	Sensitivity to hypercapnia	35% CO_2
Zandbergen et al., 1991[16]	Sensitivity to hypercapnia	35% CO_2
Gorman et al., 1994[17]	Sensitivity to hypercapnia	7% CO_2
Perna et al., 1995[18]	Compare sensitivity to hypercapnia between different psychiatric disorders	35% CO_2
Perna et al., 1995[19]	Sensitivity to hypercapnia	35% CO_2
Verburg et al., 1995[20]	Compare sensitivity to hypercapnia between different psychiatric disorders	35% CO_2
Verburg et al., 1998[21]	Compare sensitivity to hypercapnia between different psychiatric disorders	35% CO_2
Battaglia et al., 1995[22]	Sensitivity to hypercapnia	35% CO_2
Schmidt et al., 1996[23]	Sensitivity to hypercapnia	Hyperventilation
Schmidt, 1999[24]	Sensitivity to hypercapnia	35% CO_2
Welkowitz et al., 1999[25]	Sensitivity to hypercapnia, cognitive instructions	5 and 7% CO_2
Sasaki et al., 1996[26]	Sensitivity to hypercapnia	5% CO_2
Perna et al., 1994[27]	Sensitivity to hypercapnia	35% CO_2
Bocola et al., 1998[28]	Sensitivity to hypercapnia	7% CO_2
Sanderson et al., 1988[29]	Sensitivity to hypercapnia	5.5% CO_2
Verburg et al., 1998[30]	Sensitivity to hypercapnia	35% CO_2
Biber et al., 1999[31]	Sensitivity to hypercapnia	35% CO_2
Nardi et al., 2006[32]	Sensitivity to hypercapnia, PD subtypes	35% CO_2
Nardi et al., 2002[33]	Sensitivity to hypercapnia	35% CO_2
Nardi et al., 2006[34]	Compare induced and spontaneous PAs	35% CO_2
Freire et al., 2008[35]	Sensitivity to hypercapnia, PD subtypes	35% CO_2
Valenca et al., 2002[36]	Sensitivity to hypercapnia, PD subtypes	35% CO_2
Papp et al., 2001[37]	Sensitivity to hypercapnia	5% CO_2
Gorman et al., 2001[38]	Compare sensitivity to hypercapnia between different psychiatric disorders	5 and 7% CO_2
Kent et at., 2001[39]	Compare sensitivity to hypercapnia between different psychiatric disorders	5 and 7% CO_2
Blechert et al., 2010[40]	Compare sensitivity to hypercapnia between different psychiatric disorders	20% CO_2
Schutters et al., 2012[41]	Compare sensitivity to hypercapnia between different psychiatric disorders	35% CO_2
Valença et al., 2001[42]	Compare induced and spontaneous panic attacks	35% CO_2
Perna et al., 2003[43]	Sensitivity to hypercapnia	35% CO_2
van Beek et al., 2003[44]	Sensitivity to hypercapnia	35% CO_2
Niccolai et al., 2008[45]	Sensitivity to hypercapnia	35% CO_2
Rassovsky et al., 2006[46]	Sensitivity to hypercapnia	5% CO_2
Lopes et al., 2010[47]	Electroencephalogram	35% CO_2

PD = panic disorder.

1 hour before the test, and the other 10 received placebo. From 3 to 7 days, the test was repeated in a crossover design, i.e., with subjects receiving the opposite treatment (drug or placebo) to that given at the first test.

In this study, a PA was defined as a VAS-A (Visual Analogue Scale of Anxiety) score equal to or greater than 25 combined with an increase in panic symptomatology. The mean difference between the alprazolam and the placebo groups on the VAS-A was 13.3±45.6.

Nevertheless, in 1996, Sanderson et al.[52] concluded that the same acute dose (1 mg) of alprazolam could reduce anxiety and panic provoked by the inhalation of 35% CO_2. Ten PD patients received the medication 90 minutes before the test and 1 week later. The results showed a reduction rate in the number of PAs to 10% for the alprazolam group vs. 70% for placebo. Accordingly, alprazolam also reduced the number and severity of anxiogenic symptoms: using the Diagnostic Symptom Questionnaire, in which the presence and discomfort of the DSM-III-R panic symptoms experienced after the inhalation were rated on a 0-4 point scale (none, mild, moderate, severe, very severe), the mean scores were 0.31±0.39 with 1 mg of alprazolam vs. 0.88±0.62 with placebo.

Before this series of experiments with alprazolam, in 1986, Beckett et al.[53] compared the efficiency of that drug to that of another benzodiazepine, clonazepam, in a single patient with PD, and found more favorable results with the latter medication.

All of the other five studies in this section using clonazepam found in favor of its ability to prevent CO_2-triggered PAs with the 35% CO_2 challenge: Pols et al.,[54] working with 10 PD patients and a dosage range of 1 to 3 mg/d during 5 weeks; and four articles by Nardi et al. and Valença et al., assessing 6 PD patients given 2 mg/d for 10 days[55]; 22 PD patients, under an acute dose of 2 mg given 1 hour before the test[56]; 14 PD patients, given 2 mg/d for 6 weeks[57]; and 34 PD patients, given 2 mg/d for 6 weeks.[58]

In view of these findings, the authors considered clonazepam to be effective in the treatment of PD and CO_2 hypersensitivity.

Tricyclic antidepressants and SSRIs

Six articles, published from 1996 to 2002, assessed the ability of tricyclic antidepressants and SSRIs to prevent PAs in PD patients.

Pols et al.[59] exposed 11 PD patients to a 6-week fluvoxamine protocol consisting of 50 mg/d for 4 days, increasing it to 100 mg/d for the next 4 days and up to 150 to 200 mg/day for the remaining days. A 35% CO_2 challenge took place before any drug was given to the subjects, and was then repeated after fluvoxamine therapy. Using the Clinical Global Impressions Scale for Severity of Illness (CGI-S), scores at baseline and during treatment were 4.9 ± 0.53 and 3.72 ± 1.00 respectively. On the Visual Anxiety Scale (VAS-A), the decline was from 33.2 ± 31.7 to 9.5 ± 21.1. That allowed the team to conclude that the anxiogenic effect of the mixture was significantly reduced during the fluvoxamine treatment.

Other studies with tricyclic antidepressants and SSRIs conducted by the Bertani, Perna et al. group led to similar conclusions, favorable to the importance of serotoninergic modulation of CO_2 hypersensitivity. Briefly, their designs and findings were as follows. Seventy PD patients were given the tricyclic antidepressant imipramine (10 mg for the first 3 days and 20 mg for the other 4 days of the test) and the SSRIs paroxetine and sertraline (10 and 25 mg/d for the entire period, respectively)[60]; 39 PD patients received the tricyclic antidepressant clomipramine (10 mg/d for the first 3 days and 20 mg/d for the other 4 days of the test) and the SSRI fluvoxamine (50 mg/d for the entire period)[61]; 123 PD patients used the tricyclic antidepressants imipramine and clomipramine (10 mg for the first 3 days, 25 mg from the 4th to the 7th day, 25 mg from the 8th to the 14th days, and a single 25-mg capsule after 15 days), sertraline (single 25 mg dose) and fluvoxamine (single 50 mg dose)[62]; 15 PD patients used the SSRI citalopram (10 mg/d for 7 days)[63]; and 28 PD patients underwent a comparative trial of the SSRI paroxetine vs. the selective noradrenaline reuptake inhibitor (SNARI) reboxetine.[64] The results of this last experiment showed a significant reduction in reactivity to CO_2 promoted by both drugs, although the effect was more intense in the paroxetine group. That led the team to conclude that serotoninergic modulation is more relevant to hypersensitivity to CO_2.

The only exception for positive results with SSRI was reported in the experiment conducted by Coryell et al.,[65] in which 32 subjects responsive to hypercapnia undertook both a 35% CO_2 and a 5% CO_2 challenge test, followed by randomized allocation to receive a 2-week course of either 10 mg/d of escitalopram or placebo. At the end of the treatment/placebo period, tests were repeated, and the team concluded that escitalopram did not produce greater changes than placebo in panic responses or in ventilatory abnormalities seen during CO_2 exposure.

Gorman et al.[66] described an experiment with a cohort of 49 subjects. Of these, 29 had PD and 14 were healthy controls. The PD patients were given the choice between treatment with imipramine, 10 mg/d and up to 200 mg over the next 2 weeks (n=21), or a cognitive training therapy (CTT, n=8). The entire cohort was subjected to two CO_2 challenges (5 and 7%) with a 12-week interval. The authors observed a significant drop in CO_2 sensitivity in the PD group, but not in the controls.

Antipanic medication and cognitive behavioral therapy

A trial by Gorman et al.[67] reported an experiment in which 23 PD patients were subjected to a 5% CO_2 challenge test and a 7% CO_2 challenge test. The cohort was then able to choose between receiving a 12-week course of either antipanic medication or cognitive behavioral therapy (CBT), after which the CO_2 challenge would be repeated.

Fifteen of the patients chose CBT, and 10 chose medication. In the latter case, a psychiatrist prescribed individual regimens to each patient, the "best possible to achieve response." The medication regimens were as follows: four subjects received imipramine (three at 200 mg/d and one at 250 mg/d); one patient first received paroxetine and then fluvoxamine (10 mg/d and 150 mg/d, respectively); one received paroxetine alone (40 mg/d); one received sertraline (200 mg/d); one received fluoxetine alone (10 mg/d); one received fluvoxamine (150 mg/d); and one received venlafaxine (225 mg/d).

The authors did not describe differences in response to the CO_2 challenge between the CBT and medication groups, reporting only the post-treatment condition as a whole. Their conclusions attest that before treatment, 11 of 23 patients had PAs during 5% CO_2 inhalation and 14 of 22 had PAs after 7% CO_2 inhalation (one patient refused to undergo the 7% CO_2 challenge test); after treatment, 3 of 23 experienced a PA during 5% CO_2 exposure and 6 of 22 experienced an attack during 7% CO_2 exposure. Treatment with either CBT or medication was able to change the response to CO_2 inhalation. The authors also found that treatment had no significant effect on objective measures of respiratory function. Hence, they concluded that "patients felt less anxious during CO_2 inhalation after successful treatment even though treatment had virtually no effect on their ventilatory response."

Table 2 summarizes all articles reviewed in this group.

CO_2 challenge in PD patients: other studies

From 1994 to 2012, 36 articles were published reporting the use of the CO_2 challenge in PD research, observing one or more of the following criteria: no use of psychopharmaceuticals; subjects that did not have PD (relatives of PD patients or healthy population); modulation of CO_2-triggered PAs by non-psychopharmaceutical substances and procedures (exercise, ethanol, cognitive therapy, etc.).

Healthy subjects

Several experiments have sought to assess the response of healthy subjects to the CO_2 challenge and its panicogenic effects, without any sort of substance or procedure designed to prevent or minimize those reactions.

Table 2 Summary of articles from group 2

Study	Main topic	CO_2 challenge test protocol
van Den Hout et al., 1984[49]	Propanolol	35% CO_2
Woods et al., 1986[50]	Alprazolam	5% CO_2
Pols et al., 1996[51]	Alprazolam	35% CO_2
Sanderson et al., 1999[52]	Alprazolam	35% CO_2
Beckett et al., 1986[53]	Alprazolam and clonazepam	5% CO_2
Pols et al., 1991[54]	Clonazepam	35% CO_2
Nardi et al., 1999[55]	Clonazepam	35% CO_2
Nardi et al., 2000[56]	Clonazepam	35% CO_2
Valença et al., 2002[57]	Clonazepam	35% CO_2
Valença et al., 2002[58]	Clonazepam	35% CO_2
Pols et al., 1996[59]	Fluvoxamine	35% CO_2
Bertani et al., 1997[60]	Paroxetine and sertraline	35% CO_2
Perna et al., 1997[61]	Clomipramine and fluvoxamine	35% CO_2
Perna et al., 2002[62]	Imipramine, clomipramine, paroxetine, sertraline and fluoxetine	35% CO_2
Bertani et al., 2001[63]	Citalopram	35% CO_2
Perna et al., 2004[64]	Paroxetine and reboxetine	35% CO_2
Coryell et al., 2009[65]	Escitalopram	5 and 35% CO_2
Gorman et al., 1997[66]	Imipramine	5 and 7% CO_2
Gorman et al., 2004[67]	Imipramine, paroxetine, fluvoxamine, sertraline, fluoxetine, and venlafaxine	5 and 7% CO_2

This review retrieved seven articles, from 1996 to 2012, that fall into this category.

Harrington et al.[68] reported that 62 healthy volunteers (with no history of spontaneous PA) were evaluated 1 year after being subjected to the 35% CO_2 challenge so as to assess whether the test could induce spontaneous PAs in those subjects. Only six (9.7%) of the subjects reported an unexpected PA during the 12-month follow-up, which, among other data, led the authors to consider the 35% CO_2 challenge ''a safe procedure with minimal long term risk for the development of anxiety pathology.'' Details of the response of healthy subjects to different CO_2 challenge protocols have been reported by several authors. Activation of the HPA axis (cortisol levels, heart rate, and systolic blood pressure) was assessed by Argyropoulos et al.[69] using 35% CO_2, with similar results to those of Bailey et al.[70] (using 7% CO_2) and Colasanti et al.[71] The latter randomly exposed their 64 subjects to four breathing mixtures - normal air, low CO_2 (9%), medium CO_2 (17.5%), and high CO_2 (35% CO_2) - so that all subjects breathed every mixture. The results led their team to group the subjects into three distinct clusters: respiratory, neurovegetative, and cognitive, according to the most prominent symptom reported.

Two experiments by Telch et al. assessed the influence of cognitive input over response to the 35% CO_2 challenge in healthy subjects. In the first,[72] from 2011, the challenge was performed with or without the presence of a cardiac defibrillator intended to create a threat context. Prior to the test, the subjects' cardiac and anxiety sensitivity was assessed by means of questionnaires referring to specific fears and concerns. Those reporting cardiac sensitivity were more affected by the presence of the defibrillator; moreover, the authors were able to confirm the relevance of a threat context over the test. The second experiment[73] used a sort of cognitive modulation on patients before the CO_2 test. Before the test, subjects were divided in high or low anxiety sensitivity groups (high AS and low AS), according to their responses to the aforementioned questionnaire. During the instructions, one of the randomly divided groups was told that the test could ''result in various physical feelings of arousal, such rapid breathing, heart rate acceleration'' and so forth (this was called the expected arousal or EA group). The other team of subjects (the expected relaxation or ER group) was instructed that the breathing could ''result in various physical feelings of relaxation, such as lightheadedness.'' Therefore, there were effectively four groups, with the following results of PA in response to the CO_2 challenge: high AS-ER, 52%; high AS-EA, 17%; low AS-ER, 5%; and low AS-EA, 5%. It can be inferred, therefore, that dispositional tendencies potentiate the panicogenic effects of threat-relevant context variables.

Finally, Pappens et al.[74] subjected 56 healthy subjects to ambient air (control group), 7 CO_2, or 20% CO_2 to assess 'circa-strike' (fight-or-flight) responses to the test, measured by skin conductance level (which assesses the sympathetic response that stimulates sweat glands), eye blink startle, self-reported anxiety and fractional end-tidal CO_2 during inhalation of CO_2-enriched air. According to the results, ''eye blink startles were inhibited during CO_2 compared to room air breathing in both experiments (…) inhalation of CO_2-enriched air is associated with a circa-strike defensive response pattern, corroborating its application as an interoceptive, panic-relevant stimulus in fear research.''

Healthy relatives of PD patients

The search for genetic vulnerabilities to PD is greatly helped by the CO_2 challenge. The chance to provoke a symptom of a psychiatric disorder in a safe, controlled, and reproducible manner represents a unique advantage for PD research, but the scientific community did approach this test cautiously. The first CO_2 challenge study of absolutely drug-free/healthy subjects was published 11

years after the earliest CO_2 challenge experiment retrieved by this review (from 1984). That represented the first of four articles by Perna et al. with relatives of PD patients subjected to the CO_2 challenge. These experiments, all using the 35% CO_2 protocol and an exclusive cohort of first-degree relatives of patients with PD, were conducted in 1995 (n=203,[75] n=151,[76] and n=165[77]) and 1999 (n=45).[78] In general, their conclusions attest to the safety of the CO_2 challenge and its validity as a paradigm for PD research in healthy subjects with a familial vulnerability to the condition. Additionally, the authors concluded that relatives of PD patients that are responsive to CO_2 had greater odds of experiencing PAs during the CO_2 challenge than relatives of CO_2-unresponsive PD patients, and that the nR-PD subtype could even be considered a non-inherited condition.

Every other article in this section[79-84] reported similar results, using either the 35 or the 5% CO_2 protocol. The latest of these,[84] by Roberson-Nay et al., describes an experiment from 2010 in which 220 subjects with separation anxiety disorder (SAD), aged 9 to 20 years, were subjected to the 5% CO_2 challenge. The cohort was divided into four groups: 1) offspring with both SAD and PD (n=13); 2) offspring with SAD but no parental PD (n=10); 3) offspring with parental PD but not SAD (n=75); and 4) offspring with neither SAD nor parental PD (n=114). The goal was to assess whether progeny of PD patients that also have SAD exhibited greater vulnerability to the CO_2 challenge and, therefore, a greater risk of adult PD; this hypothesis was confirmed. It is important to note that this test was performed in the subjects' own homes, adding portability as another advantage of the CO_2 challenge.

Twin studies

Trials with homozygotic and dizygotic twins offer the valuable opportunity to assess the role of genetic factors in PD. Confirmation of a familial association between PD and hypersensitivity to CO_2 would allow for prevention measures that could halt the onset of PD in vulnerable relatives of patients, and perhaps even lead to genetic treatment of the disorder, if a definitive genetic marker is found. Four trials conducted from 1998 to 2012 report such screening, with a growing number of siblings tested for CO_2 hypersensitivity.

The first such study, by Bellodi et al.,[85] tested 25 pairs of dizygotic and 20 pairs of monozygotic twins, totaling 90 subjects. They reported a significantly higher concordance for PA among monozygotic twins (55.6%) than dizygotic twins (12.5%), pointing in favor of genetic factors in CO_2-induced PAs.

Battaglia et al. reported three experiments[86-88] in which another variable was added to the twins' equation: the occurrence of traumatic events such as those leading to childhood separation anxiety, death of parents, and a history of generally stressful situations in childhood. The first of these,[86] with 700 twins, suggested the existence of a continuum of childhood separation anxiety and PD, and the association of both disorders with hypersensitivity to CO_2.

Following this report, a study of 712 subjects[87] concluded that the opposite could also be true: that "genetic susceptibility to CO_2 response may influence the sensitivity to environmental hazard." The next trial,[88] also with 712 twins, suggested the existence of a relation between the occurrence of adverse life events, susceptibility to CO_2, and later development of PD.

All four studies in this section were conducted under the 35% CO_2 protocol.

Physical exercise

Three articles retrieved by this review reported that acute physical exercise routines performed just before the CO_2 challenge could reduce responsiveness to hypercapnia in PD patients and in healthy subjects.

Esquivel et al. published two of these experiments.[89,90] In the first, from 2002,[90] 20 healthy volunteers were divided into two equal groups (n=10). Before a 35% CO_2 test, one of those groups performed exercise in a bicycle ergometer for the time required to surpass a blood lactate concentration of 6 mM or until exhaustion. The remaining subjects also used the bicycle, but under minimum workload, for 12 minutes. After training, both groups rested in armchairs for 3 minutes and were subjected to the 35% CO_2 challenge. According to the authors, the first group scored a lower Panic Symptom List (PSL), but no significant differences were found in the Visual Analogue Anxiety Scale (VAAS). The difference was reported to be caused by a greater neurovegetative response by subjects, instead of psychological symptoms.

The second experiment reported by Esquivel et al.[90] was published in 2007. There, 18 PD patients were similarly divided into two groups, one performing heavy exercise and the other light exercise, both on a bicycle ergometer. Using the VAAS, the authors concluded that PD patients subjected to heavy exercise were significantly less prone to experience panic symptoms triggered by CO_2. The antipanic effects of exercise were hypothesized to be linked to serotonergic modulation of raphe structures and the action of endorphins.

Additionally, Smits et al.[91] subjected 92 healthy subjects to the 35% CO_2 challenge, with half engaging in moderate exercise (at 70% of maximum heart rate) on a treadmill and the remaining subjects resting before the test. As expected, the exercise group was less sensitive to CO_2-triggered panic.

CBT and respiratory training

The ability of CBT and/or respiratory training (RT) methods to reduce sensitivity to the panicogenic effects of CO_2 in PD patients were described by Schmidt et al.,[92] who subjected 54 PD patients to two rounds of the 35% CO_2 challenge, 12 weeks apart. After the first challenge, the subjects were divided into three groups, each exposed to one of the following experimental conditions: 1) CBT with RT; 2) CBT without RT; and 3) delayed treatment. Groups 1 and 2 received 12 treatment sessions, while group 3) received no treatment for the

12 weeks between the challenges. The authors reported that during pretreatment assessments, 74% of the patients experienced PA during the tests. After treatment, 19% of subjects in group 1 and 22% in group 2 had PAs, vs. 64% of the untreated participants of group 3. Forty-four percent of treated subjects reported no anxiety during post-treatment inhalations, vs. 0% of untreated participants.

These findings corroborate the concept that fearful responding is mediated by a catastrophic misinterpretation of bodily perturbations. Therefore, acquiring awareness and confidence that bodily sensations aroused by the CO_2 challenge were not life-threatening allowed some patients to deal with those sensations without panicking.

Meuret et al.[93] described an experiment in which 35 PD patients underwent 4 weeks of capnometry-assisted breath training, targeting respiratory deregulation. Patients with PD report a major fear of bodily sensations related with PA, such as increased heart rate and shortness of breath. The feeling of 'imminent death' associated with these bodily sensations could, therefore, be associated with the high end-tidal pCO_2 assessed in those patients. After the proposed training, the authors concluded that the "results provide little support for changes in fear of bodily sensations leading to changes in respiration, but rather suggest that breathing training targeting pCO_2 reduced fear of bodily sensations in PD."

Alcohol

According to Segui et al., "high rates of anxiety disorders, including PD, have been found in patients suffering from alcohol dependence (...) it has been suggested that alcoholic subjects with PD represent a more severe subgroup of patients."[94] Due to the importance of this comorbidity, it is of great relevance that tests of the ability of alcohol ingestion to prevent PA be conducted by the scientific community. Many PD patients might become addicted to that substance as a means of somehow managing their panic symptoms.

Two studies, using the 35% CO_2 challenge and ethanol, were included in this review. The first, from 1996, reports an experiment conducted by Kushner et al.[95] with 31 PD patients. In a single-blind procedure, all subjects were told that they would consume a moderate dose of alcohol, in form of a mixed drink. Sixteen alcoholic drinks (blood alcohol level [BAL] of 85 mg/dL) and 15 placebo (with 5 mL of vodka "squirted on top of their drink to enhance the beverage deception"), were randomly distributed to the subjects. After drinking, the subjects waited for 20 minutes before the CO_2 challenge. According to the authors, "subjects who consumed alcohol reported significantly less state anxiety both before and after the challenge; in response to the challenge, subjects who consumed alcohol experienced significantly fewer PAs when applying liberal PA criteria… [while] this effect only approached significance when applying conservative panic criteria." The liberal criteria refer to a variation of the original Acute Panic Inventory (API) that included 10 items added by Gorman in 1992, increasing test sensitivity.

In the second study, Cosci et al.[96] subjected 26 PD patients to an intravenous infusion of ethanol (BAL = 50 mg/dL) or placebo, randomly distributed during the 2 days of the test, so that every subject received both substances. The authors reported that, "compared to placebo condition, the effect of the CO_2 challenge was significantly smaller after ethanol infusion."

Regarding mechanisms of action, Kushner et al. suggested that alcohol possibly shares effects with the antipanic GABAergic drugs, besides reducing responsiveness in the locus ceruleus and interfering with cognitive processes potentially related to panic, such as self-focused attention.

Serotonin modulation

Due to apparent success of SSRI and tricyclic antidepressants in PD, experiments have been conducted to assess whether organic serotonin modulation (i.e., without the use of pharmaceuticals) would influence sensitivity to the panicogenic effects of the CO_2 challenge.

Three articles from our review describe the administration of a tryptophan (TRP)-free amino acid solution. TRP (L-tryptophan) is an essential amino acid which is converted to 5-hydroxy-tryptophan (L-5-HTP), which is in turn converted to 5-hydroxytryptamine (5-HT or serotonin), so it was expected that lower TRP would decrease availability of 5-HT and amplify the panicogenic effects of the challenge. This was confirmed by Klaasen et al.,[97] Miller et al.,[98] and Schruers et al.,[99] who verified that consumption of the TRP-free amino acid solution led to a severe depletion of TRP serum levels (of about 80%). This depletion caused no panic or anxiety before the CO_2 challenge in PD patients or healthy controls, but provoked stronger panic responses to CO_2 in patients with PD, as manifested by higher levels of subjective anxiety and neurovegetative symptoms. Controls, which did not panic under the CO_2 challenge, remained unresponsive even with lower levels of serum TRP. It is important to note that no linear correlation between TRP levels and sensitivity to CO_2 was observed - i.e., over-consumption of TRP would not prevent panic, but higher levels of serotonin might.

The fourth experiment, by Schruers et al.,[100] took a different approach by assessing the effects of an acute dose of the immediate precursor of 5-HT (L-5-HTP) over CO_2 sensitivity. Twenty-four patients with PD and the same number of controls took part in the study. They randomly received either a capsule containing 200 mg of L-5 HTP or placebo. L-5 HTP significantly reduced the reaction to the panic challenge in PD patients in terms of subjective anxiety, panic symptom score and number of PAs as compared with placebo. No such effect was observed in the healthy volunteers. L-5-HTP acts to inhibit panic, which supports a modulatory role of serotonin in PD.

Endogenous opioids

An experiment conducted by Esquivel et al.[101] described an assessment of the effect of opioids receptor blockade on sensitivity to the panicogenic effects of the CO_2

challenge. The investigators subjected 24 healthy volunteers - expected to have an "intact opioids system" - to a 35% CO_2 challenge before receiving either 50 mg of naltrexone or placebo (in a double-blinded, randomized crossover design). Naltrexone premedication alone elicited significant increments in panic-related symptoms, and responses to CO_2 were not significantly different between conditions in either group. Therefore, the authors concluded that endogenous opioids can play a role in preventing symptoms that resemble panic, but their blockade does not modify the response to hypercapnia.

Other panicogenic substances

Since the CO_2 challenge became an established protocol to provoke PA in PD patients, it has been used to measure the ability of other substances to provoke similar effects.

We selected three studies describing this sort of research. The first, by Nardi et al.,[102] compared the panicogenic effects of an acute dose of caffeine and the 35% CO_2 challenge. The relevance of this experiment is increased by the fact that caffeine is a highly consumed psychoactive substance. Nardi's test was performed with a cohort of 83 patients with PD and agoraphobia, using 480 mg of caffeine in the form of instant coffee and a caffeine-free solution as placebo. A PA was induced in 51 (61.4%) subjects during the CO_2 challenge and in 38 (45.8%) patients during the caffeine test - simply ingestion of the caffeine solution, without a subsequent CO_2 inhalation. Thirty-two patients (38.5%) did not experience a PA after any of the tests and were considered nonresponsive. All patients who had a PA during the caffeine test also had a PA during the CO_2 test. Those results allowed the team to conclude in favor of an association between R-PD subtype and hyperresponsiveness to the CO_2 and oral caffeine tests.

The second experiment with another panic-inducing substance was described by Bradwejn et al.,[103] using cholecystokinin (CCK). This substance, physiologically produced by proximal gut cells and the central nervous system, is known to stimulate responses from the pancreas and gall bladder and is involved in anxiety and satiety. In the present test, 22 PD patients received either CCK or 35% CO_2. CCK induced panic symptoms in greater number and intensity than CO_2, particularly nausea and "fear of going crazy".' Under this protocol, 91% of subjects had PAs in response to CCK, vs. 45% in response to CO_2. The investigators concluded that these agents might act on distinct systems that have a final common mechanism of action.

Table 3 Summary of articles from group 3

Authors	Main topic	CO_2 challenge test protocol
Harrington et al., 1996[68]	Healthy subjects	35% CO_2
Argyropoulos et al., 2002[69]	Healthy subjects, HPA axis	35% CO_2
Bailey et al., 2005[70]	Healthy subjects	7.5% CO_2
Colasanti et al., 2008[71]	Healthy subjects	17.5 and 35% CO_2
Telch et al., 2010[72]	Healthy subjects	35% CO_2
Telch et al., 2011[73]	Healthy subjects	35% CO_2
Pappens et al., 2012[74]	Healthy subjects	20% CO_2
Perna et al., 1995[75]	Relatives of PD patients	35% CO_2
Perna et al., 1995[76]	Relatives of PD patients	35% CO_2
Cavallini et al., 1998[77]	Relatives of PD patients	35% CO_2
Perna et al., 1999[78]	Relatives of PD patients	35% CO_2
Coryell et al., 1999[79]	Relatives of PD patients	35% CO_2
van Beek et al., 2000[80]	Relatives of PD patients	35% CO_2
Coryell et al., 2001[81]	Relatives of PD patients	5% CO_2
Pine et al., 2005[82]	Relatives of PD patients	5% CO_2
Coryell et al., 2006[83]	Relatives of PD patients	5 and 35% CO_2
Roberson-Nay et al., 2010[84]	Relatives of PD patients	5% CO_2
Bellodi et al., 1998[85]	Twins	35% CO_2
Battaglia et al., 2009[86]	Twins	35% CO_2
Spatola et al., 2010[87]	Twins	35% CO_2
Ogliari et al., 2010[88]	Twins	35% CO_2
Esquivel et al., 2002[89]	Physical exercise	35% CO_2
Esquivel et al., 2007[90]	Physical exercise	35% CO_2
Smits et al., 2009[91]	Physical exercise	35% CO_2
Schmidt et al., 1997[92]	CBT	35% CO_2
Meuret et al., 2009[93]	Respiratory exercises	Assessment of pCO_2
Kushner et al., 1996[95]	Alcohol	35% CO_2
Cosci et al., 2005[96]	Alcohol	35% CO_2
Klaassen et al., 1998[97]	Serotonin modulation	35% CO_2
Miller et al., 2000[98]	Serotonin modulation	5% CO_2
Schruers et al., 2000[99]	Serotonin modulation	35% CO_2
Schruers et al., 2002[100]	Serotonin modulation	35% CO_2
Esquivel et al., 2009[101]	Endogenous opioids	35% CO_2
Nardi et al., 2007[102]	Caffeine	35% CO_2
Bradwejn et al., 1991[103]	Cholecystokinin - panicogenic	35% CO_2
Pols et al., 1994[104]	Yohimbine - panicogenic	35% CO_2

CBT = cognitive behavioral therapy; PD = panic disorder.

Table 4 Summary of CO_2 challenge tests in reviewed articles, n (%)

Review group	Number of articles (% from total)	35% CO_2 protocol (% from group)	Other % CO_2 inhalation protocol* (% from group)	Mixed CO_2 protocols† (% from group)	Other CO_2 protocols‡ (% from group)
1	40 (43.15)	27 (65.85)	12 (29.26)	0	1 (2.43)
2	19 (20)	14 (73.68)	4 (21.05)	1 (5.26)	0
3	36 (37.89)	27 (75.00)	6 (16.67)	2 (5.56)	1 (2.78)
Total	95 (100)	68 (71.57)	22 (23.15)	3 (3.15)	2 (2.10)

* 5 to 7% and 20% CO_2 protocols.
† Combinations of the 35% CO_2 protocols and 5 to 7% CO_2 protocol.
‡ Hyperventilation and assessment of pCO_2.

Finally, yohimbine, an alpha-adrenergic, serotonin, and dopamine agonist, was tested as a potential aggravator of PAs triggered by CO_2 in an experiment reported by Pols et al.[104] In that test, 20 healthy volunteers were subjected to the 35% CO_2 challenge after receiving either 20 mg of yohimbine or placebo (double-blinded, random allocation). The results showed no significant difference of the effects of yohimbine on sensitivity to the challenge; therefore, it was not considered relevant to the anxiety response of the subjects.

Table 3 summarizes all articles reviewed in this group.

Summary of CO_2 protocols

Table 4 summarizes the CO_2 challenge test protocols used in the reviewed studies. The distinction between the 35% CO_2 protocol and the 5 or 7% CO_2 protocols is due to essential differences between the procedures involving each protocol. While the 35% CO_2 protocol is based on a single or double inhalation of the gas mixture, 5 to 7% protocols involve inhalation of the mixture for several minutes to achieve hypercapnia. The 35% CO_2 protocol was far more prevalent than other protocols in clinical and preclinical studies.

Final considerations

A substantial number of studies demonstrate that hypersensitivity to CO_2 is a valid marker of R-PD. There is also accumulated evidence that the CO_2 challenge is a useful tool for clinical and preclinical PD research, as well a safe method of inducing PAs in susceptible subjects. Furthermore, PAs triggered by hypercapnia have been described as similar to spontaneous PAs. The CO_2 challenge protocols varied among studies, mainly between CO_2 concentrations of 5% to 7% - breathed during approximately 2 minutes - and a single inhalation of 35% CO_2. The lack of a standard procedure to test sensitivity to hypercapnia hampers accurate comparisons between tests performed under different protocols, but the retrieved data allow us to consider that the 35% CO_2 challenge is becoming the standard protocol.

Many drugs with anxiolytic properties have shown positive effects in preventing PAs triggered by hypercapnia or reducing their effects, and are thus considered useful for the treatment of PD, namely benzodiazepines and SSRIs. Some studies are limited by a small number of subjects, and no conclusions could be drawn as to a definitive antipanic drug. The variety of CO_2 challenge protocols and drug dosages prevents a more accurate comparison.

Non-pharmaceutical approaches to help patients deal with panic, such as physical exercise and CBT, were proven effective by the data collected. Alcohol intake reduced the panicogenic effects of hypercapnia, which may help explain the prevalence of alcoholism among PD patients.

Screening studies of twins and relatives of PD patients subjected to the noninvasive CO_2 test demonstrated the existence of a genetic marker for PD.

Evidence indicates that the susceptibility to hypercapnia-induced PAs is a strong marker of PD, given the very low number of healthy controls that responded to the test. PD is a complex condition, given its subtypes, comorbidities, and unclear etiology. The CO_2 challenge has proven to be a useful tool for clinical and preclinical research of PD.

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Disclosure

The authors report no conflicts of interest.

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