Modulation by Muscarinic Antagonists of the Response to Carbon Dioxide Challenge in Panic Disorder

Marco Battaglia, MD; Silvana Bertella, MD; Anna Ogliari, MD; Laura Bellodi, MD; Enrico Smeraldi, MD

Background: Panic attacks can be induced in persons with panic disorder by inhalation of carbon dioxide. Hyperventilation also elicits a reflex hyperventilation, which is controlled in part by cholinergic mechanisms. This study investigated whether the exaggerated response to carbon dioxide in panic disorder (PD) can be modulated by antagonists of muscarinic cholinergic receptors.

Methods: Twelve patients with PD received biperiden hydrochloride (a muscarinic antagonist that crosses the blood-brain barrier), pirenzepine hydrochloride (a muscarinic antagonist that does not cross the blood-brain barrier), or placebo 2 hours before a 35% carbon dioxide–65% oxygen respiratory challenge (vs air as a placebo) on 3 separate days, in a double-blind, random crossover design.

Results: According to patients' self-ratings of subjective anxiety, inhalation of the carbon dioxide/oxygen mixture provoked a significant and intense response after treatment with pirenzepine and placebo. After biperiden treatment, however, hypercapnia elicited a response profile similar to that elicited by air, whereby subjective anxiety remained similar to preinhalation levels.

Conclusions: Consistent with the hypothesis of the study, a centrally active muscarinic antagonist can block the response to carbon dioxide commonly observed in subjects with PD.

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SUBJECTS AND METHODS

SUBJECTS

Subjects were recruited consecutively from outpatients of an anxiety treatment facility at San Raffaele Hospital, Milan, Italy. They had to be aged 18 through 35 years and have a clinical diagnosis of PD based on the DSM III-R, which was confirmed independently by the Mental Health Diagnostic Interview Schedule Revised. Exclusion criteria were cardiocirculatory and respiratory disorders, personal or familial history of aneurysm, hypertension (systolic blood pressure, >160 mm Hg; diastolic blood pressure, >100 mm Hg), pregnancy, epilepsy, intolerance of or hypersensitivity to biperiden or pirenzepine, glaucoma or ocular hypertension, gastrointestinal tract stenosis, megacolon or dysfunctions in gut motility, urinary retention, prostatic hypertrophy, and history of alcohol, benzodiazepine, or other drug dependence.

With these criteria, 15 subjects were recruited during 13 months. After the first or second 35% CO2–65% oxygen (O2) mixture (hereafter referred to as CO2 mixture) challenge, 3 subjects found the test excessively anxiogenic and dropped out. Therefore, this study is based on the 12 subjects who completed the protocol. Although we used the Italian version of the Mental Health Diagnostic Interview Schedule, Revised, interview, for which reliability data are available, all 12 subjects also satisfied the DSM-IV diagnosis of PD, according to reviews of interviews and clinical information. All had already been treated for PD with short half-life benzodiazepines at some time in their life. None had taken tricyclic antidepressants, selective serotonin reuptake inhibitors, or monoamine oxidase inhibitors. At the time of recruitment, 3 subjects were taking benzodiazepines (alprazolam, cloromethidiazepam, etizolam, bromazepam) at a mean daily dose of 0.5 mg alprazolam-equivalent. For these subjects, tapering was initiated, with monitoring of possible symptoms of withdrawal each second day.

In the week before the experiment, the severity of PD was assessed using the Panic-Associated Symptoms Scale, with scores ranging from 0 to 28. At initiation of the protocol, subjects had to have been free of psychotropic drugs for at least 2 weeks, and receive no medications for at least 1 week. Subjects were asked to refrain from consuming alcohol for at least 36 hours and xanthine-containing beverages for at least 8 hours, from smoking for at least 6 hours, and from eating for at least 2 hours before the tests. After complete description, an informed consent was obtained. The procedure of the CO2 mixture challenge has been approved by our hospital’s Ethical Committee. More than 600 subjects in Europe have undergone the challenge without adverse events.

PROCEDURES

All subjects received orally 100 mg of pirenzepine hydrochloride, 4 mg of biperiden hydrochloride, and placebo at 48-hour intervals before undergoing a CO2 mixture/air challenge on 3 different days, in a double-blind, random crossover design.

Pirenzepine is a muscarinic antagonist used to treat gastric ulcer. The low lipid solubility largely prevents passage of the blood-brain barrier. After oral administration, it reaches peak plasma concentrations within 2 to 2.5 hours. The mean elimination half-life is 11 hours.

Biperiden is a muscarinic antagonist used as an antiparkinsonian agent. It easily penetrates the blood-brain barrier and distributes in high concentrations to the brainstem. After oral administration, it reaches peak plasma concentrations within 2 hours, and declines biphasically, with half times of 1.5 and 24 hours.

Two hours after administration of placebo, pirenzepine, or biperiden, subjects underwent the CO2 mixture/air respiratory challenge. Once inhaled, CO2 quickly permeates the blood-brain barrier and stimulates the central nervous system. Responders experience an intense increase in anxiety, usually described as closely resembling a spontaneous panic attack and lasting from a few seconds to less than 1 minute. A receiver operating characteristic analysis showed that this challenge discriminates between patients with PD and healthy controls on the basis of subjective anxiety after the test, with a positive predictive power of 91% and a negative predictive power of 75%.

The following 2 gas mixtures were used: compressed air as placebo, and a mixture of 35% CO2 and 65% O2 mixture. Both gases were inhaled through the same self-administration mask connected to a respirometer to measure vital capacity and gas volume delivered at each inhalation.

RESULTS

There were 6 men and 6 women in the study group. Mean age was 27.35 ± 5.1 years, and mean age at onset of PD...
Subjects were informed before the challenge that they would inhale 2 different harmless gas mixtures containing different percentages of O2 and CO2, that the procedure might elicit some sensations of discomfort ranging from a few physical symptoms to a clear sensation of anxiety, and that the aim of the study was to investigate whether the drug they had received could influence their response to the challenge. The possibility that a full panic attack might take place was not mentioned, however, to avoid possible negative cognitive biases related to expectation.2,3,26,27

After vital capacity was measured, subjects inhaled 1 vital capacity of CO2 mixture, or compressed air, in a randomly assigned order, at an interval of 30 minutes between both inhalations. At the end of each inhalation, subjects were asked to hold their breath for 4 seconds. According to this standardized procedure,2,3,26,27 the test is considered valid if the subject inhales at least 80% of the vital capacity.

MEASURES

Immediately before and after each inhalation of compressed air or CO2 mixture, subjects were asked to score themselves on a Visual Analog Scale for Anxiety (VASA). Like the visual analog scales commonly used in assessments of behavioral psychotherapy,28 the VASA depicts on a 10-cm line the degree of global subjective anxiety, following a continuum from 0 (no anxiety present at all) to 100 (the worst anxiety ever imaginable), and has been used consistently and reliably in several studies of panic provocation.3,26,28-32

Likewise, subjects were asked to score themselves on the Panic Symptom List III-R (PSL-III-R), a self-rating questionnaire assessing on a 5-point scale (0 indicates absent; 100—the worst anxiety ever imaginable), and has been used consistently and reliably in several studies of panic provocation.

Evaluation of responses was made according to the following standard,2,3,26,30 procedures: global anxiety reactivity was evaluated as the percentage of maximum increment or decrement possible on the VASA scale (% VASA; range of values, −100 to 100) calculated as follows: (1) if postinhalation VASA values minus preinhalation values (% VASA) was positive, then % VASA = (VASA before inhalation) – (VASA before inhalation) 

and % PSL-III-R values obtained after the challenge in the 3 pharmacological treatments (Figure 1 and Figure 2). There was no anxious reaction to compressed air inhalation, regardless of treatment.

These responses did not differ between those patients who had (5 patients [41.7%]) vs those who had not (7 patients [58.3%]) received psychotropic medication (ie, benzodiazepine therapy) recently (Mann-Whitney test for % VASA [patients vs vs those without recent medication]: after CO2 mixture plus placebo, $U = 14.5$ [P = .63]; after CO2 mixture plus biperiden, $U = 16.0$ [P = .81]. Mann-Whitney test for % PSL-III-R: after CO2 mixture plus placebo, $U = 11.0$ [P = .29]; after CO2 mixture plus biperiden, $U = 17.0$ [P = .93]).

When blind codes were opened at the end of the experiment, the random orders of administration of gas...
mixtures (compressed air and then CO₂ mixture, or CO₂ mixture and then compressed air) across 3 different days could be arranged into 5 different sequences, whereas the orders of administration of treatments had 6 sequences. The orders of administration of gas mixtures (on 5 levels) and treatments (on 6 levels) were used for coding group memberships as independent variables in a set of Kruskall-Wallis analyses of variance that analyzed Δ% VASA and Δ% PSL-III-R after CO₂ mixture inhalation in each of the 3 treatments. Differences were nonsignificant when the responses to CO₂ mixture were grouped according to the order of administration of gas mixtures (values closest to significance observed for Δ% VASA after CO₂ mixture and pirenzepine, H₃,1₂ = 4.03 [P = .40]; for Δ% PSL-III-R, H₃,1₂ = 3.47 [P = .48]). Similarly, differences were nonsignificant when the responses to CO₂ mixture were grouped according to the order of administration of treatments (values closest to significance observed for Δ% VASA after CO₂ mixture and placebo, H₅,1₂ = 8.8 [P = .12]; for Δ% PSL-III-R, H₅,1₂ = 8.7 [P = .12]), in harmony with findings of previous studies.3,26

Qualitative responses to the experiment with CO₂ mixture yielded 9 patients (75%) and 7 patients (58%) who developed provoked panic attacks after placebo and pirenzepine, respectively, and no provoked panic attacks after biperiden, whereas there were no panic attacks with compressed air, regardless of the pharmacologic treatment (Cochran Q test, Q₃ = 39.7 [P < .001]).

The data reveal that the exaggerated response to CO₂ observed in subjects with PD can be modulated through blockade of central muscarinic receptors by biperiden. Consistently, the effect of intravenous biperiden in blocking the ventilatory response to CO₂ in normal subjects correlates with the degree of hypercapnic ventilatory response at baseline.26

It has been hypothesized4,26 that individual sensitivities to increasing concentrations of inhaled CO₂ may reflect a continuously distributed, possibly evolutionarily derived, developmental trait. The magnitude of the response to the CO₂ challenge among individuals may parallel such a distribution, with patients with PD at one extreme.4,26 It is suggested that muscarinic receptors can account at least partially for such individual variability.

It appears unlikely, however, that dysfunction of any single neurotransmitter system, or stimulation of the medullary chemoreceptor(s) by CO₂ alone, can account for the complex phenomenology of panic attacks and PD. A neuroanatomical model connecting the acute attack, anticipatory anxiety, and phobic avoidance components of PD to the brainstem, limbic system, and prefrontal cortex, respectively, has been offered.3 According to this model, stimulation of the medullary chemoreceptor(s) by CO₂ can provoke a dose-dependent increase in the firing rate of the locus ceruleus,5,35 an area with a pivotal role in anxiety,8 perhaps through the projections of the medullary nucleus reticularis gigantocellularis.5,35 Through connections of the locus ceruleus to the hippocampus and to the prefrontal cortex, other salient features of PD, such as anticipatory anxiety and phobic avoidance, can be primed and promoted5,15 after the onset of 1 or more acute attack(s) generated in the brainstem.

Some pathologic conditions also allow some links to be drawn between extreme variations in sensitivity to CO₂, muscarinic receptor function, and anxiety. In congenital central hypoventilation syndrome, which is often associated with a hypoplastic or absent arcuate nucleus at the ventral medullary surface,30 children cannot perceive ambient CO₂ variations, and have significantly less anxiety symptoms than age-matched controls.5,15 Insensitivity to the suffocation stimulus that occurs when chil-
children rebreathe their own exhalations in the prone position during sleep makes liable infants subject to sudden infant death syndrome. A significant decrease in binding of the muscarinic antagonist tritiated quinuclidinyl benzilate to muscarinic receptors in the arcuate nucleus has been demonstrated in these infants. This led researchers to hypothesize that sudden infant death syndrome is intrinsically associated with a dysfunction of muscarinic receptors in the arcuate nucleus, the same muscarinic receptors that might constitute all or part of the central chemoreceptor mediating responses to hypercapnia.

Some limitations and caveats need to be taken into account in this study. First, this is a small patient sample. Second, measures of subjective increase in anxiety have been the only index of response adopted here; assessments of ventilation would have been desirable to better delineate the role of chemosensitivity in CO₂ vulnerability.

Third, the inclusion of objective measures of peripheral anticholinergic effects would have been valuable; the central effect of biperiden as the only explanation for the different response from pirenzepine would have been more reliably interpreted in the presence of similar peripheral anticholinergic effects of biperiden and pirenzepine. Fourth, beyond double-blindedness and randomization, we have not controlled for the possible effect of patients’ expectations of the challenge. Generally, however, it has been shown that patients’ expectancy has little influence on the challenge outcome.

Fifth, although none of the subjects had spontaneously reported any effects that could be linked specifically to central cholinergic blockade (eg, drowsiness) in the 2 hours after administration of biperiden, we have not systematically controlled for possible nonspecific effects that might have contributed to blunting the response to CO₂ with this drug.

General caveats include the only partial overlap between the diagnosis of PD and CO₂-induced panic, suggesting at least partially different determinants for these 2 phenotypes.

Moreover, the 35% CO₂–65% O₂ mixture is considered a robust stimulus, but it may be less specific than lower concentrations of CO₂. Stimulation with different gas concentrations may provide different results to those reported herein, and the short-term reliability of the test is unknown.

Attenuation of the response to CO₂ mixture inhalation in patients with PD can be achieved with a variety of agents. These include tricyclic antidepressants and selective serotonin reuptake inhibitors, usually after 7 to 30 days of treatment, and benzodiazepines, but the data presented herein show very substantial reduction in responsiveness to CO₂ mixture inhalation after a single dose of biperiden, a drug which is not considered therapeutic for PD.

Although it must be remembered that the global anxiety response to laboratory provocation of panic is a complex index that is likely to be affected by different neurochemical pathways, to our knowledge this is the first report to show a specific role for acetylcholine, a neurotransmitter that is not usually associated with PD. Likewise, the ability of biperiden to modulate the response to CO₂ does not necessarily suggest an intrinsic role of cholinergic mechanisms in the pathophysiology of PD and calls for further investigations in larger samples of patient and nonpatient subjects.

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Corresponding author and reprints: Marco Battaglia, MD, Department of Neuropsychiatric Sciences, Istituto Scientifico San Raffaele Hospital, 29 via Prinetti, 20127 Milan, Italy (e-mail: marco.battaglia@hsr.it).

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