Comparison between two models of experimental anxiety in healthy volunteers and panic disorder patients

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Abstract

To further investigate the role of serotonin (5-HT) in anxiety, two tests were used in human subjects. The first was the conditioning of skin conductance response (CSCR) that associates a tone to a loud noise. The second was simulated public speaking (SPS), which is believed to represent unconditioned fear. In healthy volunteers the 5-HT_{2A} receptor blocker and 5-HT reuptake inhibitor nefazodone reduced subjective anxiety and the number of spontaneous fluctuations of skin conductance during CSCR, but enhanced anxiety induced by SPS. Opposite effects had been reported with the 5-HT releasing and uptake-inhibiting agent D-fenfluramine. Panic patients behaved like controls in the CSCR. However, they had a higher level of baseline anxiety and were insensitive to SPS. This profile resembles the reported effect of the non-selective 5-HT receptor blocker metergoline in healthy volunteers. Therefore, panic patients seem to process unconditioned fear abnormally, which may be due to lack of 5-HT inhibition in brain structures commanding flight from proximal danger stimuli. © 2002 Elsevier Science Ltd. All rights reserved.

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Evidence obtained in laboratory animals indicates that 5-HT enhances conditioned anxiety at the level of the amygdala [27]. In contrast, 5-HT seems to reduce flight (unconditioned fear) evoked by electrical stimulation of the dorsal periaqueductal gray matter (DPAG) [37]. The adaptive function of this dual action of 5-HT on brain defense systems would be to inhibit flight in situations where predatory threat is potential or remote. In such conditions, movement of the prey facilitates detection by the predator and, thus, its inhibition decreases the likelihood of predatory attack [6,15]. Regarding clinical implications, conditioned anxiety established in the amygdala has been linked to generalized anxiety disorder (GAD) whereas the extreme and stereotyped flight responses generated from the dorsal periaqueductal gray have been related to panic disorder (PD) [6,7,12–15,18,31].

This hypothesis has been tested in laboratory animals by means of the elevated T-maze, an animal model of anxiety that is intended to generate conditioned anxiety

and unconditioned fear in the same rat. This model consists of an arm enclosed by walls perpendicular to two open, elevated arms. In general, the obtained results indicate that inhibitory avoidance of the open arms is impaired by drugs that relieve GAD and enhanced by anxiogenic drugs. In contrast, one-way escape is not affected by anxiolytics and is impaired by chronic treatment with imipramine and by acute administration of fenfluramine, drugs that have been shown to improve PD (for reviews, see Refs. [16,17]).

To test the same hypothesis in human subjects, two experimental models of anxiety have been used. The first is the conditioning of skin conductance responses (CSCR) originally developed by Vila and Beech [47] and modified by Wang [48]. This procedure measures the amplitude of skin conductance responses to a tone presented ten times before (habituation) and ten times after (extinction) its pairing with a loud white noise (one-trial acquisition). The effect of tone–noise pairing is to reinstate responding to further presentation of the tone, now a conditioned stimulus (CS). This appears to involve an associative mechanism, since there was no sustained reinstatement of responding when the eleventh tone was omitted and the loud noise occurred in temporal isolation from the tones [23].

Therefore, it is assumed that this model generates conditioned anxiety, related to GAD.

The second model is the simulated public speaking (SPS) test elaborated by McNair et al. [32], in which the subject is requested to prepare a speech and then talk in front of a video camera, the performance being recorded in videotape. Anxiety and other subjective states are assessed at different phases of the experimental session through self-rating scales. Fear of speaking in public is the most common social fear found in epidemiological studies [11,42], being rather constant across gender, race and age [36]. In addition, the SPS test has been shown to provoke anxiety in healthy volunteers, irrespective of trait anxiety level, while another experimental model of anxiety, the stroop color test, was anxiogenic only in persons with high trait anxiety [35]. For these reasons, the SPS test is believed to mobilize unconditioned fear mechanisms. Although in terms of face validity SPS is similar to social anxiety disorder rather than panic, the pharmacological profile of the test seems to correlate with PD, as it will be discussed later.

The following sections present a brief review of pharmacological evidence with drugs affecting 5-HT that have been assayed in healthy volunteers. Only drugs that have been tested with both the CSCR and the SPS tests are analyzed here. Also, an investigation comparing the response of panic patients and normal controls to the same experimental models will be described.

1. Pharmacological studies in healthy volunteers

The first differential drug effects on CSCR and SPS were reported with the 5-HT$_{2A}$ antagonist ritanserin. A dose of 10 mg of the drug selectively decreased the amplitude of skin conductance responses to the tone during extinction [24] whereas the same drug treatment prolonged the rise in anxiety determined by SPS [21]. Therefore, ritanserin seems to attenuate conditioned anxiety, but to facilitate unconditioned fear. These properties correlate with the clinical actions of the drug, since the results of a double-blind, placebo-controlled study have shown 10 mg of ritanserin was as effective as 4 mg of lorazepam to improve anxiety in GAD patients [5]. In contrast, a similar study revealed that ritanserin tended to aggravate PD [10]. Accordingly, a retrospective analysis of the results of a clinical trial of ritanserin in a group of patients with several anxiety disorders found the drug to significantly worsen PD [8]. Nonetheless, it should be remarked that in the experimental studies single administration of ritanserin was used whereas in the clinical assays the drug was given chronically.

This clinical and human experimental evidence has been associated with animal findings showing that the 5-HT$_{2A}$-receptor antagonist ketanserin blocked the antiaversive effect of 5-HT when both drugs were injected into the rat DPAG [37]. This led to the suggestion that 5-HT inhibits

proximal defense (panic) by acting on 5-HT$_{2A}$ receptors in the DPAG, and that an impairment of such mechanisms could result in PD [6,15].

If these assumptions are correct, a drug that enhances 5-HT action in the DPAG is expected to decrease SPS anxiety as well as to improve PD. Before its withdrawal from the market due to cardiotoxicity, D-fenfluramine has been used to test this prediction, since this drug selectively releases 5-HT from nerve fibers that originate in the dorsal raphe nucleus and innervate the amygdala or the DPAG [38,45,46]. Furthermore, as 5-HT is believed to enhance anxiety in the amygdala D-fenfluramine is expected to facilitate CSCR.

In a study conducted by Hetem et al. [26], a group of 43 adult healthy volunteers were assigned to the SPS test and another group of 40 subjects to the CSCR test. In the SPS test, subjective anxiety (STAI) was evaluated through the visual analog mood scale (VAMS). This instrument was elaborated by Norris [34] and translated to Portuguese as well as validated by Zuardi and Karniol [49]. As expected, oral administration of 30 mg of D-fenfluramine markedly decreased the rise in anxiety caused by public speaking, the dose of 15 mg of the drug having a lesser effect (Fig. 1). Statistical analysis with MANCOVA showed that the procedure induced a significant increase in STAI along the experimental session $[F(1, 39) = 4.40, \ p = 0.001]$. MANCOVA with contrast analysis showed that the dose of 30 mg of the drug significantly decreased anxiety along the session $(F(1, 39) = 4.40, \ p = 0.043)$. In the CSCR model, however, the effect of the drug was equivocal, since the lower dose of D-fenfluramine tended, non-significantly, to increase the amplitude of the skin conductance responses during the extinction phase, but the higher dose (30 mg) was ineffective.

Regarding clinical correlation, at first sight the above results look quite puzzling, since D-fenfluramine has been reported to induce panic attacks in patients with PD [43]. However, the authors of this study pointed out that D-fenfluramine causes a slow wave of anxiety that does not resemble the sudden surge that is characteristic of a true panic attack. In the same vein, a recent study with panic patients has shown that D-fenfluramine enhances anticipatory anxiety, whereas markedly decreasing the intensity of panic attacks induced by inhalation of 5% CO_2 [33].

Contrary to the view that D-fenfluramine is a panicogenic drug, the above results together with animal evidence obtained with the elevated T-maze [16,17] suggest that D-fenfluramine should improve PD. Indeed, an open clinical trial found a therapeutic action of this drug in a group of panic patients that was resistant to conventional drug treatment [40]. Likewise, a case study by one of us [25] and the effective treatment of six additional panic patients with D-fenfluramine (L.A.B. Hetem, unpublished clinical observations) point to the same direction. Unfortunately, further studies with D-fenfluramine are not allowed after its ban from clinical use. Nevertheless, it is possible that similar