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Effects of ipsapirone and cannabidiol on human experimental anxiety

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The effects of ipsapirone and cannabidiol (CBD) on healthy volunteers submitted to a simulated public speaking (SPS) test were compared with those of the anxiolytic benzodiazepine diazepam and placebo. Four independent groups of 10 subjects received, under a double-blind design, placebo or one of the following drugs: CBD (300 mg), diazepam (10 mg) or ipsapirone (5 mg). Subjective anxiety was evaluated through the Visual Analogue Mood Scale (VAMS) and the State-trait Anxiety Inventory (STAI). The VAMS anxiety factor showed that ipsapirone attenuated SPS-induced anxiety while CBD decreased anxiety after the SPS test. Diazepam, on the other hand, was anxiolytic before and after the SPS test, but had no effect on the increase in anxiety induced by the speech test. Only ipsapirone attenuated the increase in systolic blood pressure induced by the test. Significant sedative effects were only observed with diazepam. The results suggest that ipsapirone and CBD have anxiolytic properties in human volunteers submitted to a stressful situation.

Key words: cannabidiol; diazepam; ipsapirone; anxiety; public speaking

Introduction

Benzodiazepine (BZD) compounds are the drugs most often used to treat pathological anxiety. However, their sedative effects and abuse potential have encouraged research aimed at the development of new, anxioselective drugs.

Ipsapirone is a novel 5-HT_{1a} partial agonist compound (Teicher, 1988; Marsden, 1989) that shows anxiolytic-like effects in animal tests and anxious patients (De Vry *et al.*, 1991; Kuemmel *et al.*, 1988; Beneke *et al.*, 1988). It is structurally and pharmacologically related to buspirone and gepirone, drugs that cause anxiolytic-like effects in experimental animals as well as attenuation of pathological anxiety symptoms in humans (Goa and Ward, 1986; Csanalosi *et al.*, 1987).

One characteristic of these compounds is that they are clinically effective in generalized anxiety only after several weeks of continuous administration (Kuemmel *et al.*, 1988; Beneke *et al.*, 1988), and acute anxiolytic effects, although reported in animal models (De Vry *et al.*, 1991), so far have not been demonstrated in human beings.

Reported results have shown that the decrease in body temperature, the increase in heart and respiratory rate, the decrease in response rate and the euphoria induced by the main component of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (Δ^9-THC), is antagonized by concurrent administration of cannabidiol (CBD, Carlini *et al.*, 1970; Karniol and Carlini, 1973; Karniol *et al.*, 1974; Dalton *et al.*, 1976). As regards anxiety, administration of Δ^9-THC in volunteers intensifies experimentally induced anxiety (Gregg *et al.*, 1976) and, in high doses, induces anxiety feelings alone (Malit *et al.*, 1975; Tassinari *et al.*, 1976). Zuardi *et al.* (1982) showed that the anxiogenic effect of Δ^9-THC in healthy subjects is attenuated by CBD. Since this effect does not seem to involve pharmacokinetic interactions (Agurrel *et al.*, 1981; Zuardi *et al.*, 1982), it may be due to anxiolytic properties of CBD.

Animal studies with experimental models have demonstrated effects of CBD similar to anxiolytic drugs in a conditioned emotional response in rats (Zuardi and Karniol, 1983) and in the elevated plus-maze test in both rats and mice (Guimarães *et al.*, 1990; Onaivi, Green and Martin, 1990). These results led us to hypothesize the presence of anxiolytic effects of CBD in humans.

The objective of the present experiment, therefore, is to test the acute effects of ipsapirone and CBD in healthy subjects subjected to a simulated public speaking (SPS) test, an experimentally induced anxiety test sensitive to BZD (McNair *et al.*, 1982; Guimarães *et al.*, 1987, 1989).

Material and methods

Subjects

Forty healthy subjects, 18 males and 22 females, aged between 20 and 30 years (mean = 22.8) and recruited

among university students of the Medicine or Psychology courses, participated in the study as paid volunteers. They were submitted to a clinical examination and exclusion criteria were: drug use or abuse, any physical and/or psychiatric disease, pregnancy or allergy history to any drug. Informed consent was obtained from each subject. The protocol was approved by the local Ethical Committee.

Drugs

The following drugs were employed: CBD, 300 mg (kindly supplied by Dr R. Mechoulam, Hebrew University, Israel), ipsapirone, 5 mg (kindly supplied by Dr J. Traber, Tropon, Germany) and diazepam, 10 mg (Roche, Brazil). They were packed inside identical gelatine capsules. CBD was dissolved in corn oil (100 mg/ml) while the other drugs were mixed with starch. All subjects received two capsules, one with starch and the other with corn oil.

The dose of CBD was chosen based on a previous neuroendocrinological study showing that 300 mg is able to induce changes in cortisol levels (Zuardi *et al.*, manuscript in preparation). In addition, Cunha *et al.* (1980) found anti-epileptic effects of CBD at doses ranging from 200 to 300 mg/day.

The dose of ipsapirone was chosen based on previous clinical results by Kuemmel *et al.* (1988) and Beneke *et al.* (1988) that showed anxiolytic effects of chronic administration of 2.5 and 5.0 mg/t.i.d. of ipsapirone in anxious patients.

Measurements

Subjective states were evaluated by the following self-rating scales. (1) Visual analogue mood scale (VAMS) of Norris (1971), translated into Portuguese by Zuardi and Karniol (1981). It consists of 16 analogue scales to measure drug effects. They were arbitrarily divided by Norris into four factors: anxiety, physical sedation, mental sedation and other feelings and attitudes. Due to the importance of trans-cultural influence in the correct interpretation of translated scales, we performed an initial study to confirm these divisions. Data from 90 healthy subjects (including 40 volunteers from the present study), both sexes, aged between 18 and 35 years, University students, who completed the scale under the same experimental conditions (pre-drug measure after 15 min of adaptation to the laboratory), were analysed by a factor analysis to extract any common factors which might be present (Bond and Lader, 1974). Prior to the experiment each volunteer had participated in a training section to complete the scale. (2) State-trait anxiety inventory (STAI, Spielberger, Gorsuch and Lushene, 1970), translated and validated to Portuguese by Biaggio, Natalicio and Spielberger (1976). (3) Bodily symptoms scale (BSS), a five-point scale (ranging from '0' indicating no symptoms to '4' indicating extremely marked symptoms) designed to evaluate somatic symptoms (fatigue, weakness, lethargy, headache, muscle tension, tremor, hunger, thirst, coordination difficulty, perspiration, palpitation, dyspnoea, agitation, urinary urgency, nausea, dry mouth, cloudy vision, dizziness, defaecation urgency, dysuria, paraesthesia, chest pain) which could indirectly affect anxiety. To evaluate psychomotor performance a digital-symbol substitution test (DSST) was also employed (Wechsler, 1955).

Physiological measures included blood pressure (BP) and heart rate (HR). These measurements were performed with a digital blood pressure recording apparatus (Tajiti DS-103, Japan).

Procedure

Each volunteer participated in only one experimental section. They were randomly allocated, under double-blind conditions, to receive one of the drugs or placebo. The groups were balanced in relation to sex, trait anxiety (trait $\geqslant 40$ versus trait < 40) and public speaking fear (none or very little fear versus other ratings), measured by Geer's questionnaire of fear (1965).

The SPS procedure was similar to that used by McNair *et al.* (1982) with the modifications introduced by Guimarães *et al.* (1987). Briefly, the experimental section was conducted in a sound-attenuated room, and started at 4.00 p.m. After a 15-min adaptation period, baseline measures (B) were taken followed by drug or placebo intake. After 1 h 20 min pre-stress measures (P) were performed. Immediately afterwards the subject sat in front of a videocamera and a video screen and watched a videotape with instructions about the task he/she would have to perform which was pre-recorded by one of us (F.S.G.). Subjects were told that they would have 2 min to prepare a 4-min speech about a topic he/she had learned in the previous year of their course, that would be recorded on videotape and analysed later by a psychologist. Anticipatory anxiety measurements (A) were taken before the subject started speaking in front of the camera while viewing his/her own image on the video screen. The speech was interrupted in the middle so that measurements of subjective performance anxiety (S) could be taken. Fifteen minutes after the end of speech the post-stress measures (F) were performed and the section ended.

Data analysis

Data from VAMS, STAI, DSST, BP and HR were analysed using a multivariate analysis of covariance (MANCOVA) with repeated measures to compare the groups over all points, adjusting for baseline. The factors

Table 1 Distribution of the VAMS scales according to their highest loading in the factorial analysis of results from 90 subjects

Factors	Scales	Loadings
1. Anxiety	2. Calm ..... Excited	0.79684
	10. Relaxed ..... Tense	0.79314
	8. Tranquil..... Troubled	0.74407
2. Physical sedation	9. Quick-witted..... Mentally slow	0.79269
	12. Proficient..... Incompetent	0.78759
	6. Energetic..... Lethargic	0.75456
	4. Clear-headed ..... Muzzy	0.69263
	16. Gregarious ..... Withdrawn	0.64888
	5. Well-coordinated..... Clumsy	0.64236
	3. Strong ..... Feeble	0.58922
3. Mental sedation	1. Alert ..... Drowsy	0.77782
	11. Attentive..... Dreamy	0.59093
4. Other feelings and attitudes	15. Interested ..... Bored	0.72303
	14. Amicable ..... Antagonistic	0.70477
	13. Happy ..... Sad	0.69215
	7. Contented..... Discontented	0.56842

analysed were treatment, phases and the interaction treatment × phases. A MANCOVA at each assessment point was also performed. A contrast method was used to compare each active compound with placebo (Norusis, 1988).

Individual items of the BBS were analysed at each assessment point using the Kruskal–Wallis analysis of variance for non-parametric data.

The analysis was performed with the statistical software package SPSS/PC+, version 3.0, and the level of significance was set at $p<0.05$.

Results

VAMS

The factor analysis of the 90 subjects results showed that the 16 scales could be allocated into four groups according to their highest loadings (Table 1). The distribution was similar to the one proposed by Norris (1971) and so we named the factors according to the original nomenclature.

The analysis of the grouped scales showed the following results.

Anxiety

The results are shown in Fig. 1. They showed that the procedure induced a significant increase in subjective anxiety in all groups [time factor, $F(3,108)=39.05$, $p<0.001$]. Diazepam significantly decreased subjective anxiety as compared to placebo [$F(1,35)=6.17$, $p=0.016$] during the whole trial. An analysis at each assessment point showed, in addition, that: (i) diazepam significantly decreased pre-stress [phase P, $F(1,35)=4.45$, $p=0.042$]

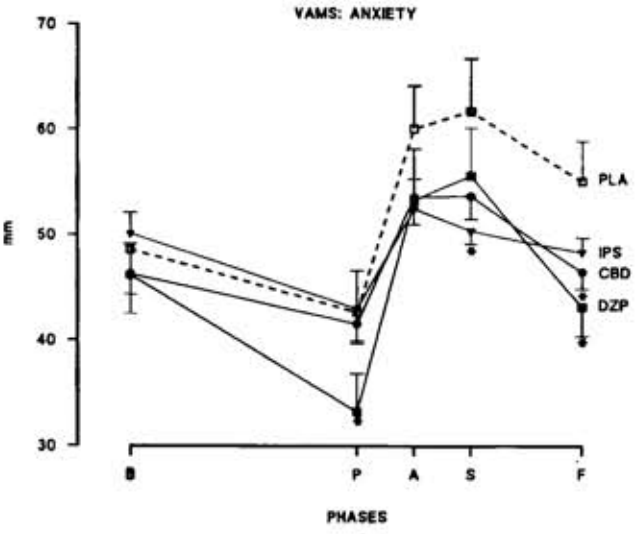

Figure 1 Effects of cannabidiol (CBD, ●), ipsapirone (IPS, ▼), diazepam (DZP, ■) and placebo (PLA, □, dashed line) on anxiety. Points are means (±SEM) of 10 healthy subjects in the following phases of the experiment: baseline (B), pre-stress (P), anticipatory anxiety (A), performance anxiety (S), post-stress (F). The asterisks indicate significant difference from placebo in each phase

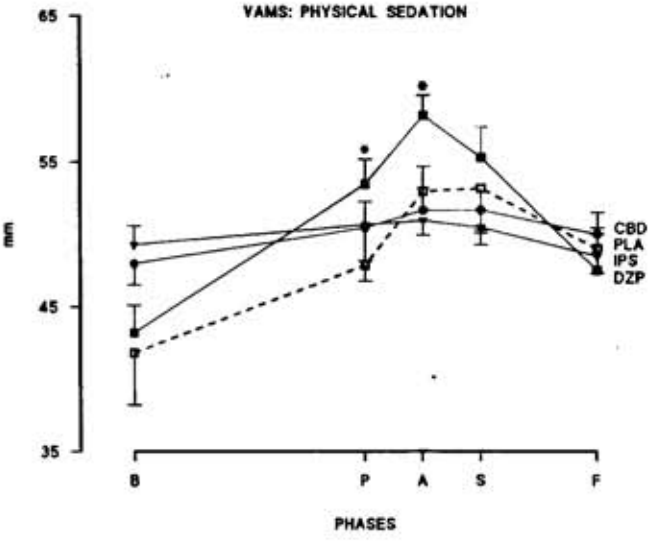

Figure 2 Effects of cannabidiol (CBD, ●), ipsapirone (IPS, ▼), diazepam (DZP, ■) and placebo (PLA, □, dashed line) on physical sedation. Further specifications as in Fig. 1

and post-stress [phase F, $F(1,35)=11.71$, $p=0.002$] anxiety; (ii) ipsapirone significantly decreased performance anxiety [phase S, $F(1,35)=4.70$, $p=0.037$] and (iii) CBD significantly decreased post-stress anxiety [phase F, $F(1,35)=6.26$, $p=0.017$].

Physical sedation

The results are given in Fig. 2. No overall drug effect was detected [$F(3,35)=1.51$, NS]. The procedure induced a

significant increase in physical sedation [time factor, $F(3,108)=7.9$, $p<0.001$]. An analysis at each phase showed that diazepam significantly increased feelings of physical sedation at pre-stress [phase P, $F(1,35)=4.75$, $p=0.036$] and anticipatory anxiety [phase A, $F(1,35)=9.84$, $p=0.003$] as compared to placebo.

Mental sedation

There was a significant decrease in mental sedation [time factor, $F(3,108)=11.54$, $p<0.001$] during the test. No significant overall drug effect was noticed [$F(3,35)=1.49$, NS] but the contrast analysis showed that diazepam tended to increase mental sedation during the trial [$F(1,35)=3.86$, $p=0.057$]. No interaction between drug × time occurred [$F(9,108)=0.70$, NS].

Other feelings and attitudes

No overall drug effect happened [$F(3,35)=0.36$, NS] but the test induced a significant increase in this rating [time factor, $F(3,108)=11.09$, $p<0.001$]. No drug × time interaction appeared [$F(9,108)=0.84$, NS].

STAI—state anxiety

No overall drug anxiety [$F(3,35)=0.47$, NS] or drug × time interaction [$F(9,108)=0.67$, NS] were found. The public speaking procedure induced a significant increase in state anxiety [time factor, $F(3,108)=51.37$, $p<0.001$].

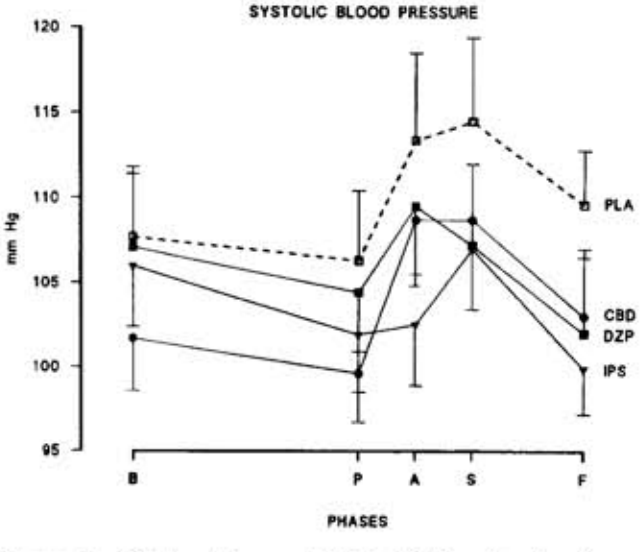

Figure 3 Effects of cannabidiol (CBD, ●), ipsapirone (IPS, ▼), diazepam (DZP, ■) and placebo (PLA, □, dashed line) on systolic blood pressure. Ipsapirone induced a significant reduction in systolic blood pressure during the trial as compared to placebo. Further specifications as in Fig. 1

Blood pressure

Results are shown in Fig. 3. The test induced an increase in systolic blood pressure levels [time factor, $F(3,108)=13.06$, $p<0.001$]. Ipsapirone reduced these levels during the experiment as compared to placebo [$F(1,35)=7.76$, $p=0.009$].

Heart rate

No overall effect of drugs [$F(3,35)=0.62$, NS] or interaction drug × time [$F(9,108)=0.63$, NS] were found.

DSST

No overall drug [$F(3,35)=0.62$, NS] or time [$F(3,108)=1.89$, NS] effects were noted.

BSS

Diazepam increased motor incoordination in phases P ($\chi^2=9.93$, $p=0.019$) and F ($\chi^2=6.82$, $p=0.077$) and dizziness in phases P ($\chi^2=9.23$, $p=0.026$) and S ($\chi^2=9.42$, $p=0.024$). No other differences emerged.

No difference among groups were found in relation to age [$F(3,35)=1.07$, NS] fear of public speaking [$F(3,35)=0.80$, NS] and trait anxiety [$F(3,35)=0.61$, NS], showing that the equalization was satisfactory.

Discussion

Confirming previous results, the SPS test induced significant increases in subjective anxiety and in its physiological concomitants (McNair *et al.*, 1982; Graeff *et al.*, 1985; Guimarães *et al.*, 1987).

Subjective anxiety differences between groups were detected by the VAMS but not by the STAI scale. This confirms earlier findings that the former scale is more sensitive in this procedure than the latter (Guimarães *et al.*, 1987, 1989).

The VAMS results showed that a single administration of ipsapirone has anxiolytic effects. These effects were detected during the anxiogenic situation, but not before. This contrasts with the anxiolytic effect of diazepam, since this drug decreased anxiety before and after the speech test (phases A and S), leaving unchanged the rise induced by it. A similar result was found with lorazepam in a previous work (Guimarães *et al.*, 1987). Although this fact conflicts with the standard psychological teaching that benzodiazepines block anticipatory anxiety, it probably reflects the unconditioned nature of the anxiety induced by performing a public speech (Deakin, Graeff and Guimarães, 1992).

The acute effect of ipsapirone in this study was unexpected since, in a previous experiment, Guimarães

et al. (1989) found no effect of 5 mg of buspirone, an ipsapirone-related compound, in healthy subjects subjected to a modified public speaking test. Also, a latency period of 2 weeks is usually needed to detect any anxiolytic effect of buspirone or ipsapirone in anxious patients (Goa and Ward, 1986; Kuemmel *et al.*, 1988; Beneke *et al.*, 1988). The lack of effect of buspirone in the work by Guimarães *et al.* (1989) may be due to a dosage problem or to a difference in procedures, since that study employed a tape recorder instead of a videocamera, making the situation less anxiogenic. It is worth noting, however, that there are pharmacological differences between these two compounds, since ipsapirone lacks affinity for D-2 receptors (De Vry *et al.*, 1991). Moreover, there is a report in the literature showing an anxiolytic effect of ipsapirone in the plus-maze model of anxiety whereas buspirone had no effect (Critchley and Handley, 1987).

Since anxiolytic effects of acute doses of ipsapirone have been reported in different pre-clinical tests (De Vry *et al.*, 1991), it is possible that our results with normal volunteers are closer to the animal models than studies with anxious patients.

The mechanism of this anxiolytic effect is still under debate. Ipsapirone acts as a fully pre-synaptic 1A receptor agonist, decreasing the firing rate of dorsal raphe neurones, and as a partial post-synaptic 1A receptor agonist (De Vry *et al.*, 1991). Deakin *et al.* (1991) and Deakin, Graeff and Guimarães (1992) have recently hypothesized that acute responses to conditioned aversive stimuli are partially mediated by 5-$HT_2/_{1c}$ receptors located in the amygdala. The anxiolytic effect of acute doses of ipsapirone may be seen, then, as resulting from a decrease in 5-$HT_2/_{1c}$-mediated neurotransmission in the amygdala due to a pre-synaptic inhibitory action. In agreement with this possibility, anxiolytic-like effects of ipsapirone microinjected into the dorsal raphe nucleus were found in the social interaction test and in the thirsty rat conflict model (Higgins, Jones and Oakley, 1987).

Even though a tolerance to inhibitory pre-synaptic effect seems to evolve with chronic 5-HT_{1a} partial agonist use, these compounds promote a down-regulation of 5-HT_2 receptors (Eison and Yocca, 1985), and thus could explain the clinical anxiolytic effect of ipsapirone. A direct, post-synaptic effect of these drugs, however, cannot be ruled out. Indeed, local application of 5-HT_{1a} ligands into the hippocampus induces anxiolytic effects in the elevated plus-maze test (Kostowski, Plaznik and Stefanski, 1989). It is possible, then, that the anxiolytic effects of 5-HT_{1a} partial agonists might involve a dual mechanism of action, both reduction in 5-$HT_2/_{1c}$-mediated neurotransmission and an augmentation of 5-HT_{1a}-mediated inhibition of limbic areas (De Vry *et al.*, 1991; Deakin *et al.*, 1991).

Ipsapirone also induced a decrease in systolic blood pressure during the test (Fig. 3). Although an anxiolytic effect of the drug could have been responsible for this, 5-HT_{1a} mechanisms are involved in central regulation of blood pressure (Saxena and Villalón, 1990). Recently, it has been proposed that cardiovascular control neurons located in the rostral part of the nucleus paragigantocellularis are under inhibitory 5-HT_{1a} modulation (Lovik, 1990).

CBD results suggest that this compound also has anxiolytic properties (Fig. 1), confirming results obtained in experiments with animals (Zuardi and Karniol, 1983; Guimarães *et al.*, 1990; Onaivi *et al.*, 1990) and in humans (Zuardi *et al.*, 1982). Similar to ipsapirone, the anxiolytic effect was not present before the anxiogenic situation.

The decrease in induced anxiety is in contrast with the anxiogenic profile of high doses of Δ^9-THC administered to volunteers (Malit *et al.*, 1975; Tassinari *et al.*, 1976; Zuardi *et al.*, 1982) and may help to understand the conflicting results obtained with *Cannabis sativa* in relation to anxiety.

Recently, binding sites for cannabinoids in the central nervous system have been demonstrated (Nye *et al.*, 1985; Herkenham *et al.*, 1990) and the structure of a putative cannabinoid receptor described (Matsuda *et al.*, 1990). So, one is tempted to speculate that these receptors might be related to anxiety.

The lack of tolerance to its anti-epileptic effects (Cunha *et al.*, 1980), of toxic effects with chronic use (Consroe *et al.*, 1991) and of Δ^9-THC-like psychotomimetic effects (Zuardi *et al.*, 1982), suggest that CBD or some derivative might be a useful clinical anxiolytic.

Only diazepam showed significant sedative effects in this experiment (Fig. 2), suggesting that the anxiolytic effects of ipsapirone and CBD cannot be attributed to a general depressant effect of these drugs.

In conclusion, the results confirm that the simulated public speaking procedure induces reliable increases in anxiety and is sensitive to drug effects. They also suggest that CBD and ipsapirone may have anxiolytic effects when administered acutely to healthy subjects.

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