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Effects of Cannabidiol (CBD) on Regional Cerebral Blood Flow

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Animal and human studies have suggested that cannabidiol (CBD) may possess anxiolytic properties, but how these effects are mediated centrally is unknown. The aim of the present study was to investigate this using functional neuroimaging. Regional cerebral blood flow (rCBF) was measured at rest using ^{99m}Tc-ECD SPECT in 10 healthy male volunteers, randomly divided into two groups of five subjects. Each subject was studied on two occasions, 1 week apart. In the first session, subjects were given an oral dose of CBD (400 mg) or placebo, in a double-blind procedure. SPECT images were acquired 90 min after drug ingestion. The Visual Analogue Mood Scale was applied to assess subjective states. In the second session, the same procedure was performed using the drug that had not been administered in the previous session. Within-subject between-condition rCBF comparisons were performed using statistical parametric mapping (SPM). CBD significantly decreased subjective anxiety and increased mental sedation, while placebo did not induce significant changes. Assessment of brain regions where anxiolytic effects of CBD were predicted *a priori* revealed two voxel clusters of significantly decreased ECD uptake in the CBD relative to the placebo condition ($p < 0.001$, uncorrected for multiple comparisons). These included a medial temporal cluster encompassing the left amygdala–hippocampal complex, extending into the hypothalamus, and a second cluster in the left posterior cingulate gyrus. There was also a cluster of greater activity with CBD than placebo in the left parahippocampal gyrus ($p < 0.001$). These results suggest that CBD has anxiolytic properties, and that these effects are mediated by an action on limbic and paralimbic brain areas.

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INTRODUCTION

Cannabidiol (CBD) constitutes up to 40% of *Cannabis sativa* (Grlie, 1976) and has quite different psychological effects to the plant's best known constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Perez-Reyes *et al*, 1973; Zuardi *et al*, 1982). In particular, in animal studies CBD has effects similar to anxiolytic drugs in conditioned emotional paradigms (Zuardi and Karniol, 1983), the Vogel conflict test (Musty *et al*, 1984), and the elevated plus maze test (Guimaraes *et al*, 1990; Onaivi *et al*, 1990). Using the latter test, anxiolytic effects were also reported for three derivates of CBD, HU-219, HU-252, and HU-291 (Guimaraes *et al*, 1994). In humans, oral administration of CBD in healthy volunteers attenuates the anxiogenic effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) (Zuardi *et al*, 1982). This effect does not seem to involve any pharmacokinetic interactions (Agurell *et al*, 1981; Zuardi *et al*, 1982), and CBD does not bind to the central known cannabinoid receptor, CB_1, (Bisogno *et al*, 2001; Mechoulam *et al*, 2002) and hence cannot be a competitive antagonist (Howlett *et al*, 1992). CBD may thus possess inherent anxiolytic properties unrelated to THC-type activity. This is consistent with its anxiolytic effect on anxiety elicited by simulated public speaking (Zuardi *et al*, 1993a).

As the receptors that mediate the psychological effects of CBD are unknown, its mechanism of action on the brain is unclear. The aim of the present study was to use functional

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neuroimaging to investigate this. In view of its anxiolytic effect, we tested the hypothesis that CBD would affect neural activity in areas that normally mediate anxiety. We compared the effects of CBD and placebo on resting cerebral regional blood flow (rCBF) in healthy volunteers in a double-blind, cross-over design. Based on previous functional imaging studies of anxiety (Maddock and Buonocore, 1997; Fischer *et al*, 1996; Liotti *et al*, 2000; Ketter *et al*, 1996), we predicted that, relative to placebo, CBD would modulate rCBF in limbic and paralimbic areas: the orbitofrontal, cingulate and medial temporal cortex, and the insula.

MATERIALS AND METHODS

Subjects

A total of 10 healthy male postgraduate students were studied. None had undergone rCBF SPECT examinations or other nuclear medicine procedures before. All were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), and were nonsmokers (of tobacco). Their mean age was 29.8 years (range 25–42 years, SD = 5.1), their mean weight was 74.1 kg (67–85 kg, SD = 6.05), and their body mass index ranged between 21 and 25 kg/m^2. The subjects had not taken any medicines for at least 3 months before the study (Mathew *et al*, 1992). No subject had a history of head trauma, neurological, or major medical illnesses, based on a semistandardized medical questionnaire and physical examination. Neither the subjects (based on the Structured Clinical Interview for DSM-IV, First *et al*, 1997) nor their first-degree relatives (based on subjects' report) had a history of psychiatric illness. No subject had used marijuana more than five times in their lives (nor in the last year), and none had used any other illegal drug. The experiment was conducted with the understanding and consent of each subject, following approval by the local ethical committee.

Cannabidiol

CBD in powder, approximately 99.9% pure (supplied by THC-Pharm, Frankfurt, Germany), was dissolved in corn oil (Zuardi *et al*, 1993a, 1995). The same amount of corn oil was used as a placebo. The drug and placebo were packed inside identical gelatin capsules.

Self-Rating Scale

Subjective states were evaluated by means of the Visual Analogue Mood Scale (VAMS) of Norris (1971), translated into Portuguese by Zuardi and Karniol (1981b). It consists of 16 analogue scales to measure drug effects, which were arbitrarily divided by Norris into four factors: anxiety, physical sedation, mental sedation, and other feelings and attitudes. A factor analysis with the Portuguese version of this scale (Zuardi *et al*, 1993a) extracted four factors that can be identified with those of the Norris proposal. Prior to the experiment, each volunteer had performed a training session completing this scale.

Procedure

Subjects were told not to consume any alcohol for 24 h and caffeine for at least 4 h before each visit to the laboratory (Mathew *et al*, 1999). Subjects who reported having less than 6 h of sleep the previous night were excluded. After at least 8 h of fasting, subjects were instructed to have a light, standardized breakfast 2 h before the experiment. They were randomly divided into two groups of five subjects. Each subject was evaluated on two different occasions, 1 week apart. In the first session, after a 30-min period of adaptation, subjects were given a single dose of oral CBD (400 mg) or placebo, in a double-blind procedure. The sessions were held in the morning (between 0800 and 1200) to minimize the effects of circadian variation. SPECT image acquisition was performed 110 min after drug ingestion. Subjective ratings on the VAMS were made 30 min before drug ingestion, at the time of drug ingestion, and at 60 min and at 75 min afterwards. In the second session, an identical procedure was followed except that the other drug was administered (ie those given CBD in the first session received placebo in the second; and *vice versa*). Subjects were informed that they would receive CBD and placebo, but they were not told in which order. The investigators were also blind to the content of the capsules.

SPECT

Subjects had a venous cannula inserted into their right arm, and rested supine with minimal environmental sensory stimulation. They were instructed to keep their eyes closed under eye pads and to relax for 15 min without falling asleep. Their ears were unplugged. VAMS ratings were made just before and 15 min after insertion of the venous cannula. At 30 min after insertion of the venous cannula, 740 MBq (20 mCi) of ethyl-cisteinate-dimer (ECD) labeled with technetium-99 m (^{99m}Tc-ECD) was injected. Subjects rested for an additional period of 5 min postinjection, after which the venous cannula was removed.

Image acquisition started 20 min after the ^{99m}Tc-ECD injection, using a double-detector SOPHA® DST system (Sophy Medical Vision, Twinsburg, USA). High-resolution low-energy collimators were used, with 128 views acquired on a 128 × 128 matrix (30 s per view), with a total acquisition time of 30 min, and approximately 75 000 counts/frame/head. Raw images were prefiltered with a Butterworth filter (order number 4, cutoff frequency 0.16), and reconstructed by filtered back-projection as transaxial slices parallel to the long axis of the temporal lobe. Attenuation correction was performed considering a pixel size of 2.55 mm and using the first-order algorithm of Chang (coefficient 0.12/cm).

Image Processing and Analysis

Images were analyzed using Statistical Parametric Mapping software (SPM99) (Friston *et al*, 1995). Reconstructed transaxial datasets were transferred to a PC (Pentium IV, 2.2 GHz, 512 Mb RAM), converted to Analyze format and reoriented to neurological convention (ie left = left).

Placebo images were realigned to CBD images using sinc interpolation. Linear (translations and rotations) and non-linear ($7 \times 8 \times 7$ nonlinear basis functions) deformations were used to register images to the SPM SPECT template, which is based on the Montreal Neurological Institute (MNI) template (Mazziotta *et al*, 1995). Finally, an isotropic Gaussian filter of 12 mm was applied to diminish inter-individual differences, and to conform data to the theory of Gaussian Random Fields (Friston *et al*, 1995), in order to allow the subsequent application of parametric statistical tests.

Between-condition (CBD *vs* placebo) comparisons of regional tracer uptake were performed on a voxel-by-voxel basis using paired *t*-tests. Before statistical testing, the regional ECD uptake of every voxel in each subject was standardized to the mean global uptake of the image in that subject, using proportional scaling. Only voxels with signal intensities above a threshold of 0.8 of the global mean (calculated using the standardized values) entered the statistical analysis. The resulting statistics at each voxel were transformed to *Z*-scores, thresholded at $Z = 2.33$ (corresponding to $p < 0.01$, one-tailed), and displayed as 3-D statistical parametric maps (SPM). These maps were first inspected for the presence of voxel clusters of significant difference in the regions where effects of CBD had been predicted *a priori* (medial temporal, cingulate, orbitofrontal, and insular cortices). Clusters in these regions were considered as significant if they included voxels with *Z*-scores of 3.09 or greater (corresponding to one-tailed $p < 0.001$), and contained more than 20 voxels. Levels of $p < 0.001$, uncorrected for multiple comparisons, have been frequently used in previous SPM analyses of positron emission tomography (PET) (Dougherty *et al*, 1999; Bremner *et al*, 1999b) and SPECT (Blackwood *et al*, 1999; Busatto *et al*, 2000) data, and are considered to provide good protection against false-positive results when there are clear hypotheses as to the location of findings. The SPMs were also inspected for differences in other, unpredicted regions. These areas were reported as significant if they survived a correction for multiple comparisons based on Gaussian random field theory ($p < 0.05$) (Friston *et al*, 1995).

For each voxel cluster showing significant between-condition differences, estimates were calculated for the mean, median, and maximal percentages of ECD count rate change (and their variances) (Table 1). These indices were obtained by partitioning the Student's *t*-test value of each voxel into its main components, with the numerator of the *t* statistic used as an approximation of the magnitude of the signal change for each contrast (placebo > CBD or CBD > placebo), and the denominator (the standard error) used to calculate the variances. The MNI coordinates for the voxels of maximal statistical significance for each anatomical brain region included in a given cluster were converted to the Talairach and Tournoux (1988) system using the method described by Brett *et al* (2002).

The four VAMS factors were submitted to an ANOVA for repeated measures in both CBD and placebo sessions. The differences between CBD and placebo in each phase of the experimental session (−30, 0, 1′00, 1′15) were analyzed by *t*-tests. Correlations between the regional tracer uptake and each of the VAMS factors scores were also investigated with SPM99, at the same statistical significance levels as described above for the between-condition rCBF comparisons. The last point in which the VAMS was applied (75′ after drug intake) was chosen for these correlations due to its proximity to the injection of the SPECT tracer. Moreover, this is the point where CBD is expected to have its maximum anxiolytic effect among all the time points chosen for assessment during the experimental session (Zuardi *et al*, 1993a). The choice for

Table 1 Limbic and Paralimbic Areas of Significant rCBF Differences in CBD Compared to Placebo Condition

Finding and cluster[a]	Cluster mean[b] and median[c] % signal change	Cluster mean[b] and median[c] variance	*P*-value (corrected)[d]	Regions included in cluster	Peak[e] % signal change (variance)	Peak[e] *Z*-score[f]	Coordinates[g]		
							x	y	z
Placebo > CBD									
Cluster 1 (102 voxels)	4.61	9.51	0.99	Left posterior cingulate cortex	4.81 (4.51)	3.40	−4	−27	47
	4.57	8.72		(BA 31)/paracentral lobule (BA5/6)					
Cluster 2 (203 voxels)	4.63	10.83	1.00	Left hypothalamus	5.61 (8.26)	3.12	−6	−6	−8
	4.56	10.53		Left amygdala–hippocampal complex /uncus	3.77 (4.53)	2.94	−16	−11	−21
CBD > Placebo									
Cluster 3 (114 voxels)	5.06 5.17	10.88 10.14	0.96	Left parahippocampal / fusiform gyri	4.53 (2.91)	3.69	−30	−15	−24

[a]Total number of voxels in each cluster that surpassed the initial threshold of $Z = 2.33$ are shown between parentheses.
[b]Average of all the voxel values in the cluster.
[c]Middle value in the distribution of frequencies of the cluster.
[d]Level of statistical significance after correction for multiple comparisons using Gaussian random field theory (Friston *et al*, 1995).
[e]Voxel of maximal statistical significance in the cluster.
[f]*Z*-score for the voxel of maximal statistical significance within each cluster.
[g]Talairach and Tornoux (1988) coordinates obtained through the conversion of SPM MNI (Mazziotta *et al*, 1995) coordinates according to Brett *et al* (2002).

this time point was also based on previous studies, which have shown that the plasma peak of an oral dose of CBD usually occurs between 1 and 2 h after ingestion (Agurell *et al*, 1981).

RESULTS

Visual Analogue Mood Scale

The administration of CBD was associated with significantly decreased subjective anxiety ($F(3,27) = 18.56$, $p < 0.001$) and increased mental sedation ($F(3,27) = 42.85$, $p < 0.001$), while placebo was not ($F(3,27) = 1.86$, $p = 0.16$ and $F(3,27) = 2.24$, $p = 0.11$, respectively) (Figure 1). In addition, an analysis at each time point indicated the following: (i) CBD was associated with significantly decreased anxiety at cannula insertion (60′ after drug intake, $t = 2.95$, $p = 0.009$) and resting phases (75′ after drug intake, $t = 5.50$, $p < 0.001$) as compared to placebo; (ii) CBD was associated with significantly increased feelings of mental sedation at cannula insertion (60′ after drug intake, $t = -3.91$, $p = 0.001$) and resting phases (75′ after drug intake, $t = -3.67$, $p = 0.002$) as compared to placebo.

Between-Condition rCBF Comparisons

The SPM showing increases in ECD uptake in the CBD relative signi

extending towards the paracentral lobule (BA5/6). At the $p < 0.001$ uncorrected level of significance, this SPM showed additional, unpredicted foci of decreased rCBF in the ECD relative to the placebo condition (> 20 voxels) in the right cerebellum, medial occipital cortex, left inferior temporal, and posterior lateral frontal cortex, but none of these

et al, 1997; Morris *et al*, 1998; LaBar *et al*, 1998; Buchel *et al*, 1998), while processing anxious faces (Breiter *et al*, 1996; Morris *et al*, 1996; Whalen *et al*, 1998; Hariri *et al*, 2002) and during pharmacologically induced anxiety (Ketter *et al*, 1996; Benkelfat *et al*, 1995; Servan-Schreiber *et al*, 1998). Functional and structural changes in the amygdala have also been reported in PTSD (Pitman *et al*, 2001; Rauch *et al*, 1996; Shin *et al*, 1997; Rauch *et al*, 2000; Liberzon *et al*, 1999), panic disorder (Uchida *et al*, 2003; Bystritsky *et al*, 2001), generalized anxiety disorder (Thomas *et al*, 2001; De Bellis *et al*, 2000), and in social (Birbaumer *et al*, 1998; Tillfors *et al*, 2001; Furmark *et al*, 2002) and simple phobias (Wik *et al*, 1997). The reduction in amygdala activity that we observed with CBD is thus consistent with the anxiolytic effect that it had in our subjects. The hippocampus has also been implicated in the processing of anxiety. Functional neuroimaging studies have shown increased activity in the hippocampus in association with anxiety in OCD (McGuire *et al*, 1994), panic disorder (Bisaga *et al*, 1998; Bystritsky *et al*, 2001; Boshuisen *et al*, 2002), PTSD (Osuch *et al*, 2001), and in social phobia (Schneider *et al*, 1999). However, other studies have reported either decreased or no difference in activity in the hippocampus in association with normal anxiety or anxiety disorders (Schuff *et al*, 2001; Schneider *et al*, 1999; Bremner *et al*, 1997; Fredrikson *et al*, 1997; Fischer *et al*, 1996; Paradiso *et al*, 1997; Liotti *et al*, 2000).

The hypothalamus is a major component of the central autonomic nervous system, and is often involved in mediating the effects of stress and anxiety (Afifi and Bergman, 1998). Functional imaging studies during fear and anxiety induction in healthy subjects (Fredrikson *et al*, 1995b; Javanmard *et al*, 1999) and in panic disorder patients (Boshuisen *et al*, 2002) have reported increases in the activity of the hypothalamic region, and hypothalamic–pituitary–adrenal axis abnormalities have been commonly reported in anxiety disorders (Hageman *et al*, 2001). The reduced hypothalamic activity that we observed is thus consistent with the anxiolytic effect of CBD.

The posterior cingulate cortex is strongly linked to temporolimbic structures (Vogt *et al*, 1992; Maddock, 1999; Afifi and Bergman, 1998), and is thought to play a central role in emotion and anxiety (MacLean, 1993; Maddock, 1999). Increased activity in the posterior cingulate gyrus has been associated with watching anxiety-provoking videos (Fischer *et al*, 1996, Fredrikson *et al*, 1995a), and with experimentally provoked obsessions and anxiety in patients with obsessive–compulsive disorder (OCD) (McGuire *et al*, 1994). Untreated patients with OCD show increased metabolism in the posterior cingulate (Perani *et al*, 1995) that decreases with treatment, with the change in posterior cingulate rCBF correlated with symptomatic improvement (Rauch *et al*, 2001, 2002). There have also been reports of increased posterior cingulate activation during symptom provocation in post-traumatic stress disorder (Bremner *et al*, 1999a) and panic disorder (Bystritsky *et al*, 2001). However, anxiety induction in phobic patients has been associated with deactivation in the posterior cingulate region (Wik *et al*, 1993) and Busatto *et al* (2000) reported a negative correlation between rCBF in the left posterior cingulate cortex and severity of symptoms in OCD.

We did not observe a correlation between the severity of anxiety and rCBF in the areas where activity was modulated by CBD, but this may have been difficult to detect because there was a 15-min gap between the points when the ratings were made and the SPECT tracer was injected.

While the areas where we found modulatory effects of CBD are thus implicated in mediating anxiety, and have also been associated with the anxiolytic effects of diazepam (Di Piero *et al*, 2001), citalopram (Van der Linden *et al*, 2000; Furmark *et al*, 2002), sertraline, and desipramine (Hoehn-Saric *et al*, 2001), these effects of CBD could be related to an effect other than on anxiety. For instance, we also observed sedative effects of CBD, confirming former findings in animals (Pickens, 1981; Monti, 1977; Colasanti *et al*, 1984; Zuardi *et al*, 1981a, 1991) and humans (Carlini *et al*, 1979; Zuardi *et al*, 1982, 1993b). This effect has been reported to be dose-related (Pickens, 1981) and CBD has also been shown to decrease wakefulness (Monti, 1977) and to cause longer sleep duration in insomniacs (Carlini and Cunha, 1981). Thus, the reduced hypothalamic activity observed after CBD use in our study could equally be related to sedative effects of CBD, as suggested to occur with other sedative compounds (Tung *et al*, 2001).

Other pharmacological effects of CBD have been reported in studies in laboratory animals and humans, such as anti-inflammatory (Malfait *et al*, 2000), anticonvulsant (Carlini *et al*, 1973; Izquierdo *et al*, 1973; Cunha *et al*, 1980), neuroprotective (Hampson *et al*, 1998), and hormonal effects (Zuardi *et al*, 1984, 1993a). In addition, the pharmacological profile of CBD is similar to that of clozapine, an 'atypical' antipsychotic drug (Zuardi *et al*, 1991, 1995), and both CBD and clozapine induce *c-fos* expression in the prefrontal cortex and lateral septal nucleus in rats (Zuardi *et al*, 2001). The mechanism(s) of action whereby CBD produces all these effects remains obscure. This is largely in contrast with the effects of Δ^9-THC, which mimics the endogenous cannabinoids in many of its actions. CBD does not act through the known cannabinoid receptors, but the stereospecificity previously observed may indicate that CBD binds to another type of receptor in the brain (Mechoulam *et al*, 2002).

In conclusion, our results suggest that CBD has anxiolytic effects that are mediated through an action on limbic and paralimbic areas of the brain. However, the findings need to be seen as preliminary, given the limitations of the study. Firstly, it would have been desirable to measure plasma levels of CBD and relate them to the magnitude of change in rCBF. Without a dose–response curve, uncertainty about the regional cerebral effects of CBD remains. Nevertheless, it should be pointed out that it is not clear whether there is a relation between plasma levels of cannabinoids—especially CBD—and their clinical effects (Agurell *et al*, 1986). In addition, the subject sample was modest and the use of SPECT limited the study's statistical power. Finally, given the limited spatial resolution of the SPECT technique and the smoothing procedure, the interpretation of large foci of tracer uptake changes as involving different brain structures of small size (such as the amygdala, hippocampus, and hypothalamus) should be made with caution. These limitations could be overcome by examining a larger sample and using functional magnetic resonance imaging, which would permit the acquisition of

greater numbers of images with a better spatial and temporal resolution.

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