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Medicinal cannabis: is Δ^9–tetrahydrocannabinol necessary for all its effects?

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Abstract

Cannabis is under clinical investigation to assess its potential for medicinal use, but the question arises as to whether there is any advantage in using cannabis extracts compared with isolated Δ^9-*trans*-tetrahydrocannabinol (Δ^9THC), the major psychoactive component. We have compared the effect of a standardized cannabis extract (SCE) with pure Δ^9THC, at matched concentrations of Δ^9THC, and also with a Δ^9THC-free extract (Δ^9THC-free SCE), using two cannabinoid-sensitive models, a mouse model of multiple sclerosis (MS), and an in-vitro rat brain slice model of epilepsy. Whilst SCE inhibited spasticity in the mouse model of MS to a comparable level, it caused a more rapid onset of muscle relaxation, and a reduction in the time to maximum effect compared with Δ^9THC alone. The Δ^9THC-free extract or cannabidiol (CBD) caused no inhibition of spasticity. However, in the in-vitro epilepsy model, in which sustained epileptiform seizures were induced by the muscarinic receptor agonist oxotremorine-M in immature rat piriform cortical brain slices, SCE was a more potent and again more rapidly-acting anticonvulsant than isolated Δ^9THC, but in this model, the Δ^9THC-free extract also exhibited anticonvulsant activity. Cannabidiol did not inhibit seizures, nor did it modulate the activity of Δ^9THC in this model. Therefore, as far as some actions of cannabis were concerned (e.g. anti-spasticity), Δ^9THC was the active constituent, which might be modified by the presence of other components. However, for other effects (e.g. anticonvulsant properties) Δ^9THC, although active, might not be necessary for the observed effect. Above all, these results demonstrated that not all of the therapeutic actions of cannabis herb might be due to the Δ^9THC content.

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Introduction

Cannabis is the third most commonly used 'recreational' drug after alcohol and tobacco (House of Lords Select Committee 1998), and there is renewed interest in the therapeutic potential of the plant *Cannabis sativa*. There are over 420 listed compounds present in the herb or resin, with even more present in cannabis smoke (Turner et al 1980; Evans 1997), but Δ^9-*trans*-tetrahydrocannabinol (Δ^9THC, formerly referred to as Δ^1THC) has long been known as the major psychoactive component (Mechoulam & Gaoni 1967). Δ^9THC mediates many of its activities through signalling via cannabinoid receptors, notably CB_1, which are expressed throughout the central nervous system (CNS) and other tissues (Devane et al 1988; Munro et al 1993; Martin 2002; Howlett et al 2002; Croxford 2003). There is a perception that marijuana can improve a number of disease-related symptoms such as pain, spasticity, spasms, muscle wasting, nausea, and hypertension in conditions such as spinal injury, multiple sclerosis, epilepsy, HIV infection, cancer and glaucoma (Consroe et al 1997; House of Lords 1998; Schnelle et al 1999; Mechoulam & Ben-Shabat 1999). Synthetically prepared Δ^9THC (known as dronabinol) has been licensed as an anti-emetic in cancer chemotherapy and to limit muscle wasting associated with HIV infection, but most of the anecdotal evidence of therapeutic activity comes from using the herb (or a whole extract of it), rather than any isolated constituent. It has been estimated that over 1% of multiple sclerosis (MS) patients take cannabis illegally for amelioration of spasticity and pain, and to improve bladder control (Consroe et al 1997; House of Lords Select Committee 1998; Williamson & Evans 2000; Pertwee 2002). There is

increasing evidence for the therapeutic potential of cannabis in this and other neurological conditions (Baker et al 2003). Most clinical studies to date are small-scale, and have used oral, isolated Δ^9THC, since smoking cannot be condoned on ethical grounds (GW Pharmaceuticals 2002; Pertwee 2002; Wade et al 2003). In contrast, MS patients tend to smoke the herb, and many claim relief of symptoms without undue psychoactive side effects (Consroe et al 1997), suggesting that even a low concentration of Δ^9THC may be effective. Oral administration of Δ^9THC causes pharmacokinetic problems for dose-titration, as it is variably absorbed and undergoes varied and significant first-pass metabolism (Agurell et al 1986; Grotenhermen 2003), and in at least one case, oral cannabis standardized according to Δ^9THC content was found to be ineffective when compared with smoking the herb (Schon et al 1999). Large controlled trials of a placebo of an oral cannabis extract matched for Δ^9THC content with dronabinol are being undertaken, but are complicated by the fact that the oral route is being used, and end points such as the Ashworth spasticity scale are subjectively-assessed, with each arm of the study using dose self-titration for each individual (Fox et al 2001). Thus, the question still remains as to whether there is any advantage or disadvantage in using cannabis herb compared with isolated Δ^9THC. Using an experimental mouse model of multiple sclerosis (MS), we have demonstrated that the cannabinoid system exhibited tonic control of spasticity, and by using intravenous administration, the effects of first-pass metabolism were avoided and anti-spasticity effects could be rapidly assessed (Baker et al 2000, 2001). CB_1 receptor agonists, including Δ^9THC, ameliorated spasticity, whereas cannabidiol (CBD), the most abundant phytocannabinoid after Δ^9THC, did not induce significant CB_1 agonism (Pertwee 1999) and was relatively ineffective. Cannabinol (CBN), a decomposition product of Δ^9THC, is a weak CB_1 agonist (Howlett et al 2002) but is normally present in significant amounts only in old samples of cannabis (Turner et al 1980; Evans 1997). CBD does however appear to antagonize CB_1 receptor agonists at prejunctional sites which are thought to be non-CB_1 or CB_2 (Pertwee 2002) and was reported to be analgesic and anti-inflammatory, acting via inhibition of cyclo-oxygenase and lipoxygenase pathways (Evans et al 1991; Mechoulam et al 2002).

It has been suggested that cannabis has potential in the treatment of epilepsy and that CBD is anticonvulsant, the potency being comparable with that of phenytoin (Carlini & Cunha 1981; Consroe 1998). It is known that epileptic patients self-medicate with cannabis (Schelle et al 1999). In addition, the mechanism of action for several anti-epileptic drugs is not known and may possibly be shown to be CB receptor-mediated in the future. There has been very little research on cannabinoids as anticonvulsants since the early 1980s and the possibility of a novel constituent of cannabis eventually being developed into a useful therapeutic compound cannot be overlooked. The piriform cortex is known to be particularly prone to epileptogenesis (Löscher & Ebert 1996) and CB_1 receptors are expressed in neurons in this brain area (Moldrich & Wenger 2000). Since other compounds present in the herb appear to modify the activity of Δ^9THC (Fairbairn & Pickens 1981; Mechoulam & Ben-Shabat 1999; McPartland & Russo 2001; Pertwee et al 2002), and both the effect and pharmacokinetics of Δ^9THC may be altered (Zuardi et al 1982; Bornheim et al 1995; Bornheim & Reid 1999), we decided to compare the effects of a standardized cannabis extract (SCE) with pure Δ^9THC, and with an extract devoid of Δ^9THC (Δ^9THC-free SCE). To do this we have used two cannabinoid-sensitive models: anti-spastic effects in an in-vivo model of multiple sclerosis using chronic relapsing experimental allergic encephalomyelitis (CREAE) mice, and an in-vitro seizure model of epilepsy in immature rat piriform cortical brain slices.

Materials and Methods

Chemicals

Mexican *Cannabis sativa* L. was grown hydroponically, under licence from the Home Office, UK. Δ^9THC and CBD were purchased from Sigma-Aldrich (Poole, UK). Oxotremorine-M was purchased from Semat Ltd (St Albans, UK). SR141716A was kindly provided by NIDA/NIH (Bethesda, MD). The cannabinoids and SR141716A were dissolved in ethanol for anticonvulsant work (final bath concentration of ethanol not exceeding 0.001%, a concentration of ethanol which applied alone under control conditions produced no noticeable effect), or ethanol cremophore and phosphate-buffered saline (PBS) (1:1:18) before injection in the spasticity model. Oxotremorine-M was prepared as a 10 mM stock solution in distilled water and stored at 4 °C; dilutions were freshly prepared in Krebs solution and bath-applied by superfusion (bath-exchange time ~30 s).

Plant material and preparation of extracts

Cannabis extracts were prepared by Soxhlet extraction of the freshly dried herb with hexane. The dried extract was dissolved in ethanol, filtered and assayed by HPLC to identify and quantify the Δ^9THC content using a Waters XTerra C_{18} 5 μm column and an acetonitrile/water gradient. This extract was dried under vacuum and re-dissolved in a suitable volume of ethanol, to produce an extract containing 20% Δ^9THC (Figure 1A), designated the standardized cannabis extract (SCE). This sample of cannabis contained very little CBD and CBN, as can be seen in Figure 1A (where no large peaks occur in the vicinity of the Δ^9THC peak). A Δ^9THC-free extract (Δ^9THC-free SCE) was prepared by subjecting a proportion of the SCE to preparative HPLC, during which the Δ^9THC was removed from the eluate of successive injections, after identification on a Waters 2787 Dual wavelength detector. The process was repeated with a similar amount of the SCE but the Δ^9THC was not removed, this fraction was designated the 'treated standardized cannabis extract' (TSCE), and was done to allow a comparison to be made and evaluate any variation which may have been caused by passage through the HPLC process. Confirmation that the Δ^9THC had been removed was made by comparison of spectra on analytical

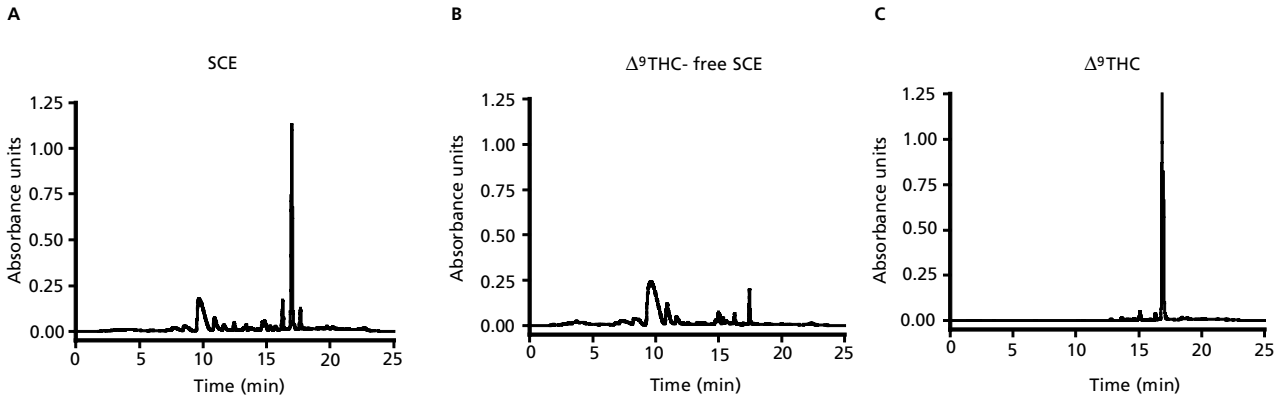

Figure 1 HPLC chromatograms of cannabis extracts used. Conditions are as specified in the methods. A. SCE (standardized cannabis extract); the major peak is Δ^9THC. B. Δ^9THC-free SCE, showing no significant peak corresponding to Δ^9THC. C. Pure Δ^9THC. Major peak is due to Δ^9THC.

HPLC (Figure 1B). The peak corresponding to Δ^9THC is shown in Figure 1C.

In-vivo anti-spastic effects in CREAE model of multiple sclerosis

Chronic relapsing experimental allergic encephalomyelitis (CREAE) was induced in ABH mice following immunization with mouse spinal cord homogenate in Freund's adjuvant. Animals were monitored for the development of spasticity, typically 60–80 days post-inoculation. Progressive spasticity develops after a number of paralytic relapse episodes, and cannot yet be modelled in-vitro. Spasticity was assessed by measuring the resistance forces to bend the hind limb to full flexion against a strain gauge at baseline following intravenous (i.v.) injection of cannabinoids (Baker et al 2000). Differences following treatment were analysed using pair wise, repeated measure, one-way analysis of variance, using the Student–Newman Keuls method as reported by Baker et al (2001). The statistics were performed using Sigmastat software, which included a test for normality and equality of variance. In comparison with synthetic full CB_1 agonists, which exhibit a rapid (within 10 min) anti-spastic effect (Baker et al 2000, 2001), Δ^9THC, which is only a partial CB_1 agonist (Pertwee 1999), is typically slower acting even when injected intravenously (Baker et al 2000). This dose was not maximally active compared with full agonists or higher doses of Δ^9THC, but still significantly inhibited spasticity. The effect was measured over 60 min, as we had previously shown that the effect of Δ^9THC was maximal within an hour (Baker et al 2000).

Inhibition of induced epileptiform activity in immature rat piriform cortical brain slices

An in-vitro seizure model of epilepsy, utilizing immature rat piriform cortical (PC) brain slices, induced by bath-application of the muscarinic agonist oxotremorine-M (10 μM) (Postlethwaite et al 1998), was used to evaluate potential anticonvulsant properties of bath-applied Δ^9THC, SCE, TSCE and Δ^9THC-free SCE. This in-vitro brain slice model (Postlethwaite et al 1998) has a well known neuronal circuitry, and can be regarded as a model of *status epilepticus* limbic epilepsy in man, where there is self-sustaining recurrent or continuous seizure activity (Löscher & Ebert 1996). Animals were decapitated after deep halothane anaesthesia in accordance with the Home Office Animals (Scientific Procedures) Act 1986. Preparation and maintenance of brain slices in standard Krebs medium and subsequent intracellular microelectrode recording was carried out as described by Constanti et al (1993). Δ^9THC, SCE, TSCE and Δ^9THC-free SCE were examined. We tested CBD alone and then together with Δ^9THC in the ratio of 70:30, on the brain slices only. The reason for this was the reputed anticonvulsant properties of CBD and the reported modifying effects on Δ^9THC (Bornheim et al 1995; Consroe 1998; Pertwee et al 2002). This particular ratio was chosen for the preliminary experiments as it was within the normal range found commonly in cannabis herb (Turner et al 1980). The time required to abolish oxotremorine-M-induced bursting firing was recorded, together with any associated changes in neuronal resting membrane potential and input resistance. Differences between groups were assessed using Student's *t*-test.

Results

Inhibition of spasticity in an experimental model of multiple sclerosis in-vivo

After Δ^9THC injection, spasticity returned within a few hours; one week later the same group of animals was injected with 5 mg kg^{-1} SCE intravenously (containing 20% Δ^9THC) and this was also anti-spastic compared with baseline. Whilst SCE appeared to inhibit spasticity to a comparable level with that obtained with pure Δ^9THC, what was particularly noticeable was that SCE

caused a more rapid onset of muscle relaxation, and a reduction in the time to maximum effect than with Δ^9THC alone (Figure 2A). As can be seen in Figure 2, the inhibition of spasticity by Δ^9THC had a rather slow development. This was similar to that seen by Baker et al (2000), where the reduction of spasticity was comparable with that observed with the SCE in this instance, despite using a 10-fold lesser concentration of drug. However, the slope of the curve following administration of the cannabis extract consistently demonstrated a rapid inhibition of spasticity within 10 min. Previously, we had tested animals with different levels of spasticity and in each case the shape of the curve was similar (Baker et al 2000). To ascertain whether Δ^9THC was essential for activity, a Δ^9THC-free SCE was tested in a similar manner. As before, 5 mg kg^{-1} SCE injected intravenously significantly inhibited spasticity, but when the 5 mg kg^{-1} (i.v.) Δ^9THC-free SCE was subsequently tested in the same way, no significant reduction in spasticity was produced (Figure 2B). Although Δ^9THC or SCE were administered to the same animals more than once, and tolerance to the CB_1 receptor can occur, the animals treated in Figure 2B were treated 24 h apart, with the first dose being Δ^9THC-free. Therefore, desensitization of the receptor could not have occurred due to Δ^9THC. The effect of the treated extract (TSCE, containing 5 mg kg^{-1} i.v. pure Δ^9THC) produced an inhibitory effect on spasticity in the mice in a manner similar to that of the SCE. This indicated that passage through the HPLC column had not affected activity and confirmed that the presence of Δ^9THC was necessary for the anti-spastic effect of cannabis in the model (Figure 2B).

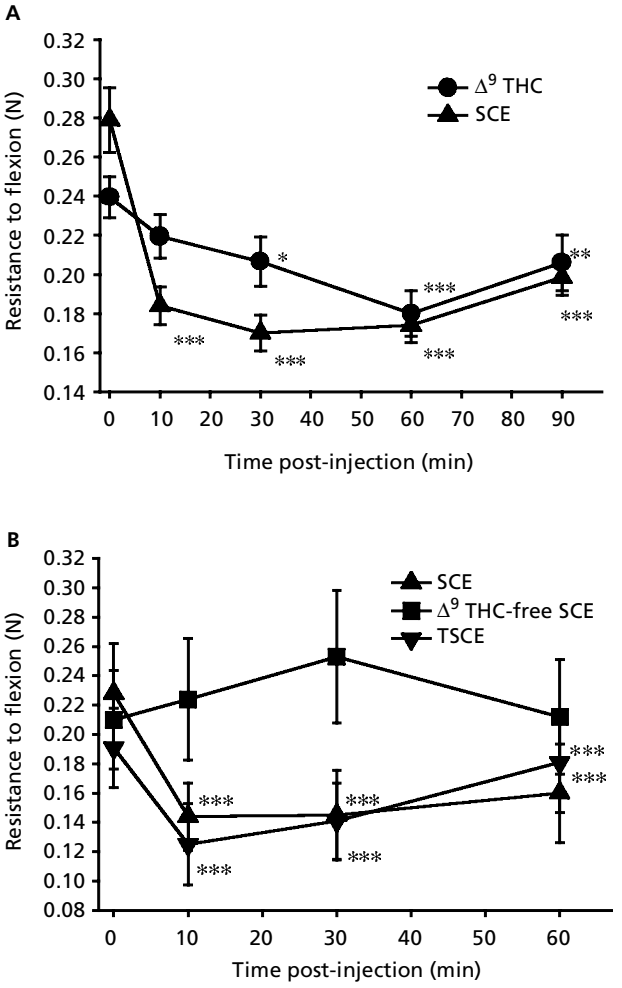

Figure 2 Effect of various cannabis extracts compared with isolated Δ^9THC on spasticity in an in-vivo model of multiple sclerosis. Following the induction of chronic relapsing experimental allergic encephalomyelitis, spasticity of the hind limbs developed. This was measured by the resistance to full flexion of the hind limbs against a strain gauge before and following intravenous administration of (A) 1 mg kg^{-1} Δ^9THC, and one week later in the same group of animals (n = 8 mice) with 5 mg kg^{-1} SCE containing 20% Δ^9THC in vehicle or (B) 5 mg kg^{-1} SCE, Δ^9THC-free SCE and TSCE in the same group of animals (n = 6 mice), separated by at least 48 h. The data points represent means ± s.e.m. of resistance force (Newtons, N) of 12 individual spastic hind limbs in each experiment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ are significantly different means compared with baseline control of individual experiments.

Inhibition of in-vitro oxotremorine-M-induced epileptiform activity in brain slices of immature piriform cortex

In this in-vitro seizure model of epilepsy, SCE and TSCE (both 0.3 μg mL^{-1}, equivalent to 1 μM Δ^9THC) consistently and rapidly abolished the epileptiform bursting activity induced by bath-applied oxotremorine-M (SCE: mean onset time = 280 ± 60 s; n = 8; Figure 3A; TSCE: mean onset time = 270 ± 75 s; n = 5). Δ^9THC (1 μM; n = 6) took significantly longer to abolish bursting activity (mean time for onset = 840 ± 120 s; $P < 0.05$ vs 0.3 μg mL^{-1} SCE; Figure 3B). However, in all instances following Δ^9THC, bursting activity was replaced by intermittent spiking and low amplitude oscillations in membrane potential, accompanied by a periodic increase in membrane 'noise'. CBD alone (1 μM, n = 4) had no effect upon oxotremorine-M-induced bursting activity (effect indistinguishable from that produced before addition of drug; trace not shown). To examine whether CBD might be modulating the effect of Δ^9THC in this system, an arbitrarily-selected 70:30 (Δ^9THC: CBD) mixture was tested but showed essentially the same effect as 1 μM Δ^9THC alone (mean onset time = 780 ± 110 s; $P > 0.05$ vs 1 μM Δ^9THC; n = 5; not shown). Interestingly, Δ^9THC-free SCE (0.3 μg mL^{-1}) was also found to abolish epileptiform activity in a manner indistinguishable from that seen on application of SCE (mean onset time = 260 ± 75 s; $P > 0.05$ vs 0.3 μg mL^{-1} SCE; n = 8; not shown). This result indicated for the first time that there was a cannabinoid component(s) in the SCE (other than Δ^9THC and CBD) which possessed anticonvulsant activity. The fact that SCE and Δ^9THC-free SCE had very similar burst-blocking activity could therefore reflect a mutual antagonism between this component(s) and Δ^9THC itself in the natural state. At present, further speculation as to the detailed mechanism of action is inappropriate because of the number of

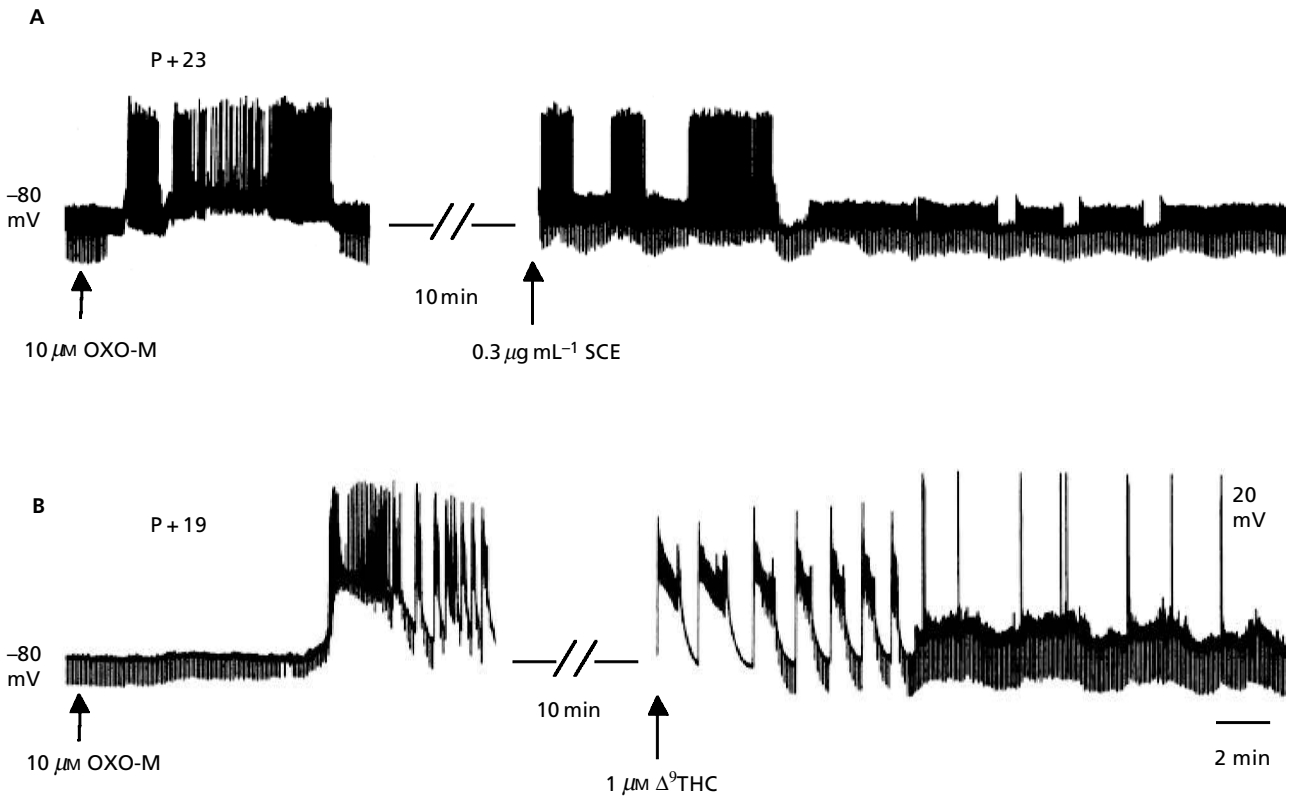

Figure 3 A. Continuous chart trace of intracellular membrane potential, showing the effect of SCE (0.3 μg mL^{-1}) on oxotremorine-M (OXO-M)-induced epileptiform burst firing induced in the immature rat piriform cortical slice preparation. SCE applied to an immature (P + 23) presumed deep pyramidal neuron exhibiting established bursting behaviour in 10 μM oxotremorine-M abolished epileptiform bursting and hyperpolarized the cell membrane back to control resting level (-80 mV). B. (Different neuron.) Chart trace showing the effect of Δ^9THC (1 μM) on established oxotremorine-M-induced epileptiform burst firing induced in the immature rat piriform cortical slice preparation. Δ^9THC applied to an immature (P + 19) presumed deep pyramidal neuron in the presence of 10 μM oxotremorine-M only partially abolished epileptiform bursting. The membrane potential remained slightly depolarized and the cell input resistance remained greater compared with controls. Note also that the membrane potential did not return to control values (−77 to −73 mV), cell input resistance remained elevated and residual slow wave-like behaviour (characteristic of a subthreshold bursting neuron) was also apparent following Δ^9THC application.

components present in the mixture. It may be noted that none of the agents tested fully reversed the oxotremorine-M-induced depolarization of the cell membrane or the associated increase in cell input resistance, therefore were unlikely to be acting via an antimuscarinic mechanism.

On cell membrane properties, 1 μM Δ^9THC applied alone consistently produced a small hyperpolarization (3 ± 1 mV%; $P < 0.05$ vs control; n = 4) and a reduced input resistance (12 ± 2.1%; $P < 0.05$ vs control; n = 4) in a reversible manner; however, rather surprisingly, 0.3 μg mL^{-1} SCE or 0.3 μg mL^{-1} Δ^9THC-free SCE increased input resistance (SCE: 24 ± 3.3%; $P < 0.05$ vs controls; n = 4; Δ^9THC-free SCE: 22 ± 4.1%; $P < 0.05$ vs controls; n = 4) with little change in membrane potential. The effects of Δ^9THC, SCE, TSCE and Δ^9THC-free SCE (n = 3 experiments for each extract) were all fully blocked by the CB_1 receptor antagonist SR141716A (1 μM), pre-applied for 20 min to the bathing medium, thereby confirming their CB_1 receptor-mediated activity. SR141716A 1 μM alone had no effect on membrane properties or oxotremorine-M-induced bursting behaviour. These results indicated that for equivalent concentrations of Δ^9THC, the SCE was a more potent anticonvulsant than isolated Δ^9THC; even the Δ^9THC-free SCE showed substantial antiseizure activity. Finally, CBD (1 μM, n = 3) did not exhibit any anticonvulsant activity, nor did it modulate the effects of Δ^9THC, or affect neuronal membrane properties in this model.

Discussion

This study has demonstrated that there were clear differences between the effects of pure Δ^9THC and a total extract of the cannabis herb containing a matched dose of Δ^9THC, and that a cannabis extract without Δ^9THC still had anticonvulsant, but not anti-spastic, activity, at the doses tested. There is increasing support to suggest that medical cannabis extracts can inhibit spasms and spasticity in multiple sclerosis and spinal cord injury (Baker et al 2003; Wade et al 2003; Croxford & Miller 2003), however at least in this experimental paradigm of spasticity, the active component was

Δ^9THC. This is the major CB_1 agonist in cannabis, accounting for most of its cannabimimetic effects (Howlett et al 2002), and is consistent with a central role of CB_1 receptors and the endogenous cannabinoid system in regulating the severity of this sign (Baker et al 2000, 2001). This finding implied that the therapeutic action of cannabis in spasticity would be invariably linked with some psychoactive effects, as both are controlled by the same receptor, and cannabis has no mechanism to preferentially target individual brain regions (Howlett et al 2002). Therefore, the clinical outcome would be a balance between symptom control and side effects in any particular condition and some people would inevitably find these intolerable and discontinue the drug (Robson 2001; Wade et al 2003). Nevertheless, reports (particularly using a sublingual spray) appear to indicate that a useable therapeutic window exists, and many patients do not experience debilitating psychoactive effects (Brady et al 2002; Notcutt 2000; GW Pharmaceuticals 2002). Our data suggested that cannabis contained more than just Δ^9THC in its therapeutic armoury and these other compounds might contribute to the various actions of Δ^9THC. A simple explanation might be that cannabis contains a host of additional compounds that could stimulate, albeit weakly, the CB_1 receptor. CBN has this property (Pertwee 1999), but the use of fresh herb minimizes concentration levels of CBN. Likewise Δ^8THC, an isomer of Δ^9THC (also a CB_1 agonist and psychoactive agent) was not present in SCE as it co-eluted with, and was therefore removed with, the Δ^9THC. In a recent, small scale, blinded clinical trial, the incidence of 'mild' adverse events was higher in MS patients taking an oral preparation of the plant extract than those taking an equivalent dose of Δ^9THC (Killestein et al 2002), which would be consistent with additional CB_1 activity. In addition, cannabinoids might contribute to stimulation of this pathway through indirect mechanisms, by affecting degradation of endocannabinoids from the synaptic cleft, for which CBD has reputed activity (Bisogno et al 2001; Jacobsson & Fowler 2001); likewise, components of cannabis might stimulate other receptors that were in a homeostatic feedback with the cannabinoid system (Wilson & Nicoll 2001). Alternatively, other components of cannabis might contribute to enhanced pharmacokinetics, CNS permeability or alter tolerability. For example, CBD ameliorated clinical anxiety provoked by pure Δ^9THC (Zuardi et al 1982), increased levels of Δ^9THC in the brain of mice after administration of both drugs (Bornheim et al 1999), and blocked CYP 450 3A11, the enzyme responsible for hydroxylation of Δ^9THC to the 11-hydroxy metabolite, a more potent CB_1 agonist (Bornheim et al 1995; Pertwee 1999). It is generally thought that the ratio of the concentrations of CBD and Δ^9THC in the extract determines many of the therapeutic benefits of the herb, particularly pain relief (Notcutt et al 2000). It is also possible, but unlikely, that a different concentration ratio of Δ^9THC:CBD might interact in some way. The Δ^9THC:CBD mixture was only analysed in the in-vitro epilepsy model and not the CREAE mouse model, since although Δ^9THC has been analysed in spasticity and was found to be efficacious, CBD has been tested and was found to be ineffective (Baker et al 2000). The results from this study showed that if Δ^9THC was removed from the cannabis then the anti-spastic effect was lost, indicating that CBD in the SCE was not significantly anti-spastic. It has been indicated in the literature that CBD might be anticonvulsive (Carlini et al 1981; Consroe 1998), so more emphasis was placed on this aspect and our limited supply of CBD was used for the in-vitro brain slice work. However, our results did not support an anticonvulsive role for CBD itself, nor did it modify the anticonvulsive activity of Δ^9THC against muscarinic agonist-induced epileptiform bursting in the piriform cortex. We have shown that although the anticonvulsive effects of the SCE at least partly resided in the Δ^9THC content of the herb, there was nevertheless at least one other factor present in the SCE (which may or may not be another cannabinoid) that exerted an anticonvulsive effect via a CB_1-mediated mechanism (Turner et al 1980), acting independently or in concert with one another. Cannabis has been reported to have both pro- and anti-symptomatic effects (House of Lords Select Committee 1998; Consroe 1998) and the finding that cannabinoids can regulate both excitory and inhibitory neurotransmitters (Howlett et al 2002; Wilson & Nicoll 2001) suggested the outcome could depend on both the location of the CB_1 receptors within the neural control circuit affected and the specificity of the components of the extract for those receptors.

Conclusions

This work suggested that medicinal preparations of cannabis should be characterized chemically to a greater degree than simply specifying the concentration of Δ^9THC and CBD, to maximize efficacy and minimize side effects. It supported the subjective reports by cannabis users (whether for medicinal or illicit purposes) that marijuana might have a different effect to that of isolated Δ^9THC, although this might be reflective of differences in route of administration (Hart et al 2002). As far as some properties of cannabis are concerned (e.g. anti-spasticity), we concluded that Δ^9THC was the active constituent, although its effects might be modified by the presence of other components. However, for other effects (e.g. anticonvulsant properties) Δ^9THC, although active in itself, has been shown to be not the only constituent with anticonvulsant properties, although surprisingly, this work did not support previous reports that CBD had anticonvulsive activity (Consroe 1998). The fact that the CB_1 receptor antagonist SR141716A blocked the anticonvulsant effects of the extracts indicated a CB_1 receptor-mediated mechanism of action, confirming the work of Wallace et al (2002), where the endocannabinoid anandamide was shown to be anticonvulsant in a whole animal mouse model. Although Δ^9THC-free SCE has been shown to act through CB_1 receptors, this does not necessarily confer psychoactivity amongst its effects, which depends on receptor affinity, degree of agonism and CB_1 receptor location in particular brain areas. For example, CBN has some affinity for CB_1 receptors, but is devoid of psychoactivity, and it has been shown that even CB_2 agonists bind to CB_1 receptors but do not agonize the receptor (Howlett et al 2002). There is also the possible involvement of novel CB

receptors, the existence of which has recently been postulated and to some extent characterized (Breivogel et al 2001; Hajos et al 2001; Monory et al 2002). However, it is not possible or appropriate to discuss these findings in relation to these, or other receptors with cannabimimetic effects (Brooks et al 2002) or which can stimulate endocannabinoid release (Wilson & Nicoll 2001), or mechanisms by which the endocannabinoid system may be inactivated or otherwise modified (Guiffrida et al 2001) until the factor(s) present in the herb has been isolated and identified. Above all, these results demonstrated that the therapeutic actions of cannabis herb were not exclusively due to the Δ^9THC content. This has important implications for drug development, in that it might be possible to separate psychoactivity from anticonvulsant, but not anti-spastic, effects. The pharmacology of the cannabinoids is highly complex, and the involvement of other non-cannabinoid components in a plant extract makes this even more so, but these results lend support to the idea that the therapeutic use of cannabis has a greater potential than using pure Δ^9THC alone. It also suggests that other non-psychoactive compounds might be discovered, and by selecting a suitable ratio of constituents it might also be possible to minimize side effects and increase efficacy. It is therefore likely that preparations containing different cannabinoid ratios will be developed for different therapeutic indications in the future, but determining the effective composition of these will require further basic research, in addition to clinical investigation.

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