Euphytica **140:** 73–82, 2004.
© 2004 *Kluwer Academic Publishers. Printed in the Netherlands.*

Pharmacological and therapeutic targets for Δ^9-tetrahydrocannabinol and cannabidiol

Roger G. Pertwee
School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, U.K.; (e-mail: rgp@abdn.ac.uk)

Key words: cannabidiol, cannabinoid receptors, clinical applications of cannabinoids, pharmacological actions of cannabinoids, tetrahydrocannabinol, tolerance to cannabinoids

Summary

Cannabis is the unique source of a set of at least 66 compounds known collectively as cannabinoids. Of these, most is known about the pharmacology of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of cannabis, and about cannabidiol (CBD), which lacks psychoactivity. Accordingly, this paper focuses on the pharmacological and therapeutic targets of these two cannabinoids. Many of the effects of Δ^9-THC are mediated by cannabinoid receptors of which at least two types, CB_1 and CB_2, are present in mammalian tissues. Endogenous agonists for cannabinoid receptors have also been discovered. CB_1 receptors are present at the terminals of central and peripheral neurones, where they modulate transmitter release. They also exist in some non-neuronal cells. CB_2 receptors are expressed mainly by immune cells, one of their roles being to alter cytokine release. Δ^9-THC also appears to have non-CB_1, non-CB_2 pharmacological targets. It is already licensed for clinical use in the U.S.A. as an anti-emetic and appetite stimulant and both Δ^9-THC and Δ^9-THC-rich cannabis extracts show therapeutic potential as neuroprotective and anticancer agents and for the management of glaucoma, pain and various kinds of motor dysfunction associated, for example, with multiple sclerosis and spinal cord injury. CBD has much less affinity for CB_1 and CB_2 receptors than Δ^9-THC and its pharmacological actions have been less well characterized. Potential clinical applications of CBD and CBD-rich cannabis extracts include the production of anti-inflammatory and neuroprotective effects, the management of epilepsy, anxiety disorders, glaucoma and nausea, and the modulation of some effects of Δ^9-THC.

Abbreviations: CBD: cannabidiol, THC: tetrahydrocannabinol

Introduction

Cannabis sativa is the unique source of a set of more than 60 oxygen-containing aromatic hydrocarbon compounds known collectively as cannabinoids (Table 1). It also contains a number of other compounds of potential interest, including at least 120 different terpenes and 21 flavonoids (reviewed in ElSohly, 2002). Of these cannabis constituents, most is known about the pharmacology of two of the cannabinoids: (–)-Δ^9-tetrahydrocannabinol (Δ^9-THC; Figure 1), which is psychoactive, and (–)-cannabidiol (CBD; Figure 1) which is not (reviewed in Grotenhermen, 2002; Iversen, 2000, 2003; Paton & Pertwee, 1973a, 1973b; Pertwee, 1988, 2004c, 2004d). This paper focuses on the actions and therapeutic potential of these two cannabinoids.

Pharmacological targets for Δ^9-THC

Since Δ^9-THC has high lipid solubility and low water solubility, it was long thought to owe its pharmacological properties to an ability to perturb the phospholipid constituents of biological membranes in

Table 1. Cannabinoid constituents of *Cannabis*[a]

Cannabinoid type	Number[b]
Δ^9-tetrahydrocannabinol	9
Δ^8-tetrahydrocannabinol	2
Cannabidiol	7
Cannabigerol	6
Cannabichromene	5
Cannabicyclol	3
Cannabielsoin	5
Cannabitriol	9
Miscellaneous	11
Cannabinol[c]	>1
Cannabinodiol[c]	>1

Reviewed in ElSohly (2002).
[a]Plant cannabinoids are also known as phytocannabinoids.
[b]Number of cannabinoids of this type.
[c]Cannabinol and cannabinodiol are probably air-oxidation artifacts derived from tetrahydrocannabinol and cannabidiol respectively.

a structure-dependent manner (reviewed in Pertwee, 1988). Whilst it remains possible that this is one of its actions, research begun in the 1980's has now firmly established that mammalian tissues contain at least two

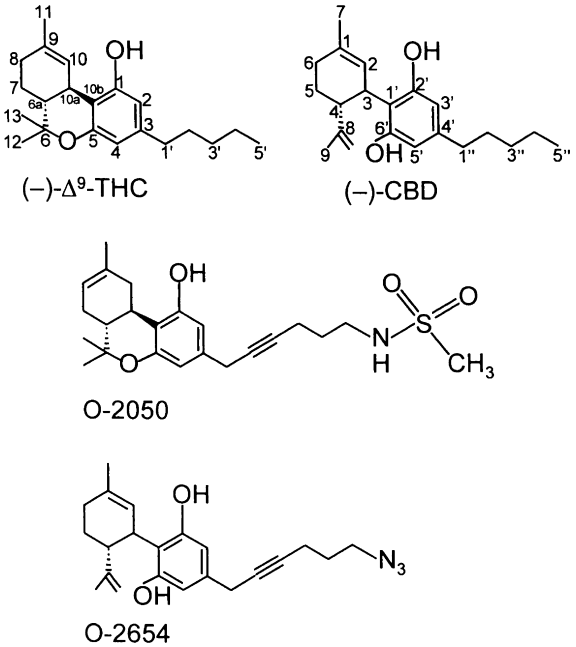

Figure 1. The structures of the plant cannabinoids, (–)-Δ^9-tetrahydrocannabinol ((–)-Δ^9-THC) and (–)-cannabidiol ((–)-CBD), and of synthetic analogues of (–)-Δ^8-THC (O-2050) and (–)-CBD (O-2654) (see the section on Bioassays for Δ^9-THC and CBD).

Table 2. Cannabinoid CB_1 and CB_2 receptors

Receptor type	CB_1	CB_2
Cloned	Yes	Yes
Coupled to G proteins	Yes	Yes
Some effector systems identified	Yes	Yes
Selective agonists	Yes	Yes
Selective antagonists/inverse agonists	Yes	Yes
Endogenous agonists	Yes	Yes
Present in brain and spinal neurones	Yes	No
Present in some peripheral neurones	Yes	No
Present at some nerve terminals	Yes	No
Modulates neurotransmitter release	Yes	No
Present in other cell types (e.g. immune cells)	Yes	Yes

Reviewed in Howlett et al. (2002).

types of pharmacological receptor for Δ^9-THC. These are cannabinoid CB_1 and CB_2 receptors (Table 2) (reviewed in Howlett et al., 2002; Pertwee, 1997). Δ^9-THC acts as a partial agonist at both these receptor types, exhibiting lower CB_2 than CB_1 efficacy. Indeed, it is presumably because of its rather low CB_2 efficacy that Δ^9-THC has been found to behave as a CB_2 receptor antagonist in at least one *in vitro* bioassay system (Bayewitch et al., 1996).

CB_1 and CB_2 receptors are both coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinase. CB_1 receptors are also coupled through $G_{i/o}$ proteins to certain ion channels, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q type calcium channels and to D-type potassium channels. CB_1 receptors can also act through G_s proteins to activate adenylate cyclase. Additional signalling mechanisms for cannabinoid CB_1 and CB_2 receptors have been proposed and descriptions of these can be found elsewhere (Howlett et al., 2002).

CB_1 receptors are present in the central nervous system and also in some peripheral tissues including pituitary gland, immune cells, reproductive tissues, gastrointestinal tissues, sympathetic ganglia, heart, lung, urinary bladder and adrenal gland (reviewed in Howlett et al., 2002; Pertwee, 1997). Many CB_1 receptors are to be found at central and peripheral nerve terminals and an important function of these receptors is to suppress the release of a range of neurotransmitters (Howlett et al., 2002). Much less is known about the role of CB_2 receptors although it is very likely that this includes immunomodulation as CB_2 receptors are

expressed mainly by immune cells, particularly B-cells and natural killer cells (reviewed in Howlett et al., 2002; Pertwee, 1997). One important role of CB_2 receptors may be to regulate cytokine release in health or disease (Howlett et al., 2002; Molina-Holgado et al., 1999). If this is true, then a common property of CB_1 and CB_2 receptors would be the ability to modulate ongoing release of various chemical messengers, CB_1 receptors from neurones and CB_2 receptors from immune cells.

Within the brain, the distribution of CB_1 receptors is heterogeneous, brain areas that express this receptor type including the cerebral cortex, hippocampus, caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus, cerebellum, periaqueductal grey, rostral ventromedial medulla, superior colliculus and certain nuclei of the thalamus and amygdala (Herkenham et al., 1991; Pertwee, 1997, 2001). This distribution pattern accounts for several prominent pharmacological properties of Δ^9-THC, for example their ability to impair cognition and memory and to alter the control of motor function. It also accounts for the ability of these agonists to produce analgesia in humans and antinociception in animal models both of acute pain and of tonic pain induced by nerve damage or by the injection of an inflammatory agent. More specifically, as detailed elsewhere (Pertwee, 2001), CB_1 receptors that mediate the analgesic/antinociceptive effects of cannabinoids seem to be located not only in the brain but also on the terminals of neurones that project from the brain stem to the spinal cord and/or on intrinsic spinal neurones. There are also CB_1 receptors at the central and peripheral terminals of primary afferent neurones, both on small diameter C-fibres and on larger diameter Aβ and/or Aδ-fibres. The presence of significant numbers of CB_1 receptors on these larger diameter primary afferent fibres helps to explain the efficacy shown by CB_1 receptor agonists against signs of neuropathic pain in animals since this kind of pain is thought to be elicited in part by abnormal spontaneous discharges of myelinated Aβ- and Aδ-fibres. CB_2 receptors, and possibly other types of cannabinoid receptors yet to be characterized, may also contribute to the analgesic/antinociceptive effects of cannabinoids (Hanus et al., 1999; Hohmann et al., 2004; Pertwee, 2001; Quartilho et al., 2003).

CB_1 and CB_2 receptors also serve as targets for endogenous agonists, all of which are eicosanoids (reviewed in Howlett et al., 2002; van der Stelt & Di Marzo, 2004). Among these "endocannabinoids" are arachidonoylethanolamide (anandamide) and 2-arachidonoyl glycerol, both of which are synthesized on demand rather than stored. Following their release these endocannabinoids enter cells by a combination of simple diffusion and facilitated, carrier-mediated transport (reviewed in Hillard & Jarrahian, 2003). They are then metabolized by intracellular enzymes, anandamide by fatty acid amide hydrolase and 2-arachidonoyl glycerol mainly by monoacylglycerol lipase (monoglyceride lipase) (reviewed in Cravatt & Lichtman, 2002; Dinh et al., 2002; Ueda, 2002; van der Stelt & Di Marzo, 2004). Endocannabinoids together with cannabinoid receptors constitute what is now usually referred to as the "endocannabinoid system."

Whilst there is little doubt that CB_1 receptors mediate many of the central effects of Δ^9-THC that are listed in Table 3, there is also evidence for the existence of other non-CB_1, non-CB_2 pharmacological targets for this cannabinoid (reviewed in Pertwee, 2004a). These are listed below.

- Non-CB_1, non-CB_2, non-TRPV1 (vanilloid VR1) receptors on capsaicin-sensitive perivascular sensory neurons that induce the release of calcitonin gene-related peptide when activated (Zygmunt et al., 2002). These may be ANKTMI ion channels which, like TRPV1 receptors, belong to the transient receptor potential (TRP) family of ion channels, are implicated in the detection of noxious cold and appear to be insensitive both to the CB_1 receptor agonists, HU-210 and CP55940, and to the endocannabinoids, anandamide and 2-arachidonoyl glycerol (Jordt et al., 2004).
- A novel CB_1 receptor subtype in spinal cord though which Δ^9-THC can induce antinoception in rats and mice (reviewed in Howlett et al., 2002; Pertwee, 2001).
- Allosteric sites on 5-HT_3 receptors at which Δ^9-THC inhibits 5-HT_3 receptor currents (reviewed in Pertwee, 2004a) with greater potency (EC_{50} = 38.4 nM) than synthetic cannabinoids that are more potent than Δ^9-THC as CB_1 or CB_2 receptor agonists (Barann et al., 2002).
- Sites at which Δ^9-THC (and CBD) inhibit delayed rectifier potassium currents (Mamas & Terrar, 1998; Poling et al., 1996).
- Sites on neuronal transporters of dopamine and noradrenaline at which transport is enhanced by low nanomolar concentrations of Δ^9-THC and inhibited by higher concentrations of this cannabinoid (reviewed in Pertwee, 1988).

Table 3. Some *in vivo* effects in man of Δ^9-THC or of cannabis that are most probably attributable to Δ^9-THC

Effects that have therapeutic potential
Analgesia, including relief from neuropathic and inflammatory pain
Effects on motor function, including relief from muscle spasms and spasticity
Neuroprotection
Inhibitory effects on gastro-intestinal tract motility
Anti-emetic effect
Reduction of intra-ocular pressure
Facilitation of sleep
Appetite stimulation
Inhibitory effect on cancer cell proliferation
Effects contributing to the "high"
Elevation of mood
Laughter
Loquacity
Effects on perception[a]
Feelings of increased insight and significance
Other effects
Impairment of cognition, learning and memory
Impairment of the ability to concentrate
Impaired psychomotor performance; ataxia; tremor
Sense of unreality, depersonalization and detachment
Fragmentation of thoughts
Feelings of panic or anxiety; dysphoria
Production/exacerbation of psychotic symptoms; paranoia
Effects on cardiovascular function including tachycardia and postural hypotension
Conjunctival reddening, reduced tear flow; dry mouth
Nausea and occasional vomiting
Effects on endocrine and reproductive function
Effects on thermoregulation

For references see Grotenhermen (2002), Iversen (2000), Murphy (2002), Paton & Pertwee (1973a), Pertwee (1985) and sections on Pharmacological targets for Δ^9-THC, Δ^9-THC and CBD are both neuroprotective agents and Therapeutic targets for Δ^9-THC and CBD.

[a]For example, colours appear more vivid, music seems more pleasant and felt time passes more slowly than clock time.

- Sites on neuronal transporters of 5-HT, γ-aminobutyric acid and choline at which Δ^9-THC augments or inhibits transport (reviewed in Pertwee, 1988).
- Binding sites on catecholamine and benzodiazepine receptors (reviewed in Pertwee, 1988).
- Phospholipase A_2 which can be activated by Δ^9-THC (reviewed in Pertwee, 1988).
- Cyclo-oxygenase and monoamine oxidase which are inhibited by Δ^9-THC (reviewed in Pertwee, 1988).
- Membrane phospholipids (reviewed in Pertwee, 1988).

The extent to which these proposed non-CB_1, non-CB_2 targets contribute to particular pharmacological effects produced by Δ^9-THC remains to be established.

Pharmacological targets for CBD

CBD is of interest because it produces several pharmacological effects which may come to have clinical applications (Table 4). It is unlikely that CB_1 and CB_2 receptors play a major role in the production of these effects as CBD has much lower affinity for these receptors than Δ^9-THC, and as it appears to have other pharmacological targets with which it interacts more

Table 4. Some *in vivo* effects of CBD

Effect
Anxiolytic
Anticonvulsant
Anti-inflammatory
Neuroprotective
Anti-emetic
Inhibitory effect on L-DOPA-induced dystonia
Prolongation of pentobarbitone- and hexobarbitone-induced sleep[a]
Induction of the microsomal enzymes, CYP2B, CYP2C and CYP3A
Attenuation of some effects of Δ^9-THC[b]
Enhancement of some effects of Δ^9-THC[c]
Antipsychotic[d]
Appetite suppressant[d]
Sleep-promoting effect[d]
Inhibitory effect on cancer cell proliferation[d]
Reduction of intra-ocular pressure[d]

L-DOPA, L-dihydroxyphenylalanine.
For references see Pertwee (2004c,d).
[a]Through the inhibition of cytochrome P450 (CYP) enzymes.
[b]For example, Δ^9-THC-induced "high", feelings of panic or anxiety and impairment of the ability to concentrate in human subjects and Δ^9-THC-induced aggression and convulsions in animals.
[c]For example, Δ^9-THC-induced inhibition of intestinal motility in mice and Δ^9-THC-induced reductions of food and water intake in rats.
[d]Only limited evidence.

readily (Table 5). Still to be established, however, is the extent to which these and/or as yet unidentified targets for CBD mediate each of the effects listed in Table 4. Interestingly, (+)-CBD has greater CB_1 and CB_2 affinity than its natural (–)-enantiomer, as do certain structural analogues of (+)- and (–)-CBD (reviewed in Pertwee, 2004c).

Δ^9-THC and CBD are both neuroprotective agents

As discussed in greater detail elsewhere, Δ^9-THC and CBD both possess anti-oxidant (electron donor) activity that is sufficient to protect neurones against oxidative stress associated, for example, with glutamate-induced excitotoxity (El-Remessy et al., 2003; Fowler, 2003; Hampson et al., 1998; Hampson et al., 2000; Marsicano et al., 2002; Mechoulam et al., 2002; Pertwee, 2004c, 2004d; Platt & Drysdale, 2004; van der Stelt et al., 2002). This property is shared by other cannabinoids that contain a phenol group, irrespective of whether or not they bind to CB_1 or CB_2 receptors, whilst several non-phenolic cannabinoid receptor agonists have been found to lack anti-oxidant activity (Marsicano et al., 2002). Δ^9-THC and CBD may also induce neuroprotection through additional mechanisms, for example, Δ^9-THC by acting through presynaptic receptors to inhibit glutamate release from neurones and/or calcium entry into neurones through N and P/Q type channels (Fowler, 2003; Mechoulam et al., 2002; Pertwee, 2004a; van der Stelt et al., 2002) and CBD (1 μM) by opposing the release of calcium from intracellular stores stimulated by metabotropic glutamate receptor activation (Drysdale et al., 2004). As well as possessing neuroprotective activity, Δ^9-THC and CBD have the ability to induce signs of programmed (apoptotic) or unprogrammed (necrotic) cell death in some biological systems (Downer et al., 2003; Drysdale et al., 2004; Gallily et al., 2003; McKallip et al., 2002; Platt & Drysdale, 2004).

Bioassays for Δ^9-THC and CBD

For Δ^9-THC, the most commonly used *in vivo* bioassay is the mouse tetrad, in which its ability to produce hypokinesia, hypothermia, catalepsy in the Pertwee ring test and antinociception in the tail-flick or hot plate test is determined in the same animal (reviewed in Howlett et al., 2002; Martin et al., 1995; Pertwee, 2004b).

Table 5. Some putative pharmacological targets for CBD

Targets	Effective CBD concentrations
	Up to 1 μM
Mitogen-induced cytokine release (modulation)	0.032 to 64 μM
Reactive oxygen species (anti-oxidant activity)	0.1 μM
CYP (P450) enzymes (inhibition)	0.1 μM[a]
Neuronal calcium channels (blockade)	0.1 to 1 μM
Cell viability processes (enhancement and inhibition)	0.1 to 8 μM
Neuronal target in the mouse vas deferens (antagonism of cannabinoid CB_1 receptor agonists)	0.12 μM[b]
Glucocorticoid receptors (displacement of ^{3}H-dexamethasone)	0.2 μM[c]
Receptors for abnormal-CBD on microglial cells (modulation of migration)	0.3 μM
Membrane phospholipids (perturbation)	~0.3 μM
Cardiac L-type calcium channels (blockade)	0.32 μM
Cardiac delayed rectifier potassium channels (blockade)	0.32 μM
Neuronal transporters of catecholamines & 5-HT (inhibition)	1 μM
Allosteric (?) site on neuronal metabotropic glutamate receptors (inhibition)	1 μM[d]
Allosteric (?) site on α_1-adrenoreceptors of vas deferens smooth muscle (inhibition)	1 μM
	>1 to 10 μM
Receptors for abnormal-CBD on endothelial cells (modulation of vasomotor tone)	>1 μM
Binding to cannabinoid CB_1 and CB_2 receptors	>1 μM
Lipoxygenases (inhibition)	2.9 μM[c]
Oestradiol receptors (displacement of ^{3}H-oestradiol)	5.6 μM
TRPV1 (vanilloid) receptor activation	3.5 μM[c]
Phospholipase A_2 (activation)	6.4 μM[c]
Neuronal transporter of γ-aminobutyric acid (inhibition)	10 μM
Neuronal transporter of choline (inhibition)	16 μM[c] Above10 μM
Anandamide transporter (inhibition)	22 μM[c]
Fatty acid amide hydrolase[e] (inhibition)	27.5 μM[c]
Dopamine receptors (displacement of ^{3}H-spiperone by various ligands)	30 μM
Cyclo-oxygenase (inhibition)	39.8 μM[c]

See also Pertwee (2004c,d) for references.

[a]In most experiments, CBD has been reported to inhibit CYP enzymes at concentrations in the micromolar range.

[b]K_B value for surmountable antagonism of the CB_1/CB_2 receptor agonist, *R*-(+)-WIN55212 (Pertwee et al., 2002).

[c]EC_{50} value.

[d]Drysdale et al. (2004).

[e]Fatty acid amide hydrolase metabolizes the endocannabinoid, anandamide (see the section on Pharmacological targets for Δ^9-THC).

Other *in vivo* bioassays for Δ^9-THC include the dog static-ataxia test, the monkey behavioural test, the rat catalepsy test and the drug discrimination test (Howlett et al., 2002; Martin et al., 1995; Pertwee, 2004b). As to established *in vitro* bioassays for Δ^9-THC and for other CB_1 and CB_2 receptor agonists, these all involve the use of membrane or tissue preparations that contain CB_1 and/or CB_2 receptors, expressed either naturally or after transfection (reviewed in Howlett et al., 2002; Pertwee, 1997, 2004b). The most commonly used *in vitro* assays are listed below.

- Binding assays that measure the ability of Δ^9-THC to displace radiolabelled cannabinoid receptor ligands from membranes obtained from CB_1 and/or CB_2 receptor-expressing cells or tissues.
- The [^{35}S]guanosine-5′-*O*-(3-thiotriphosphate) ([^{35}S]GTPγS) binding assay that can measure both CB_1 and CB_2 receptor mediated-stimulation of binding to G proteins of the hydrolysis-resistant GTP analogue, [^{35}S]GTPγS.
- The cyclic AMP assay that relies on CB_1 and CB_2 receptor-mediated inhibition (usual effect) or enhancement of basal or drug-induced cyclic AMP production.
- An assay that measures the ability of CB_1 and CB_2 receptors to mediate the production of increases in intracellular free Ca^{2+} levels.
- An assay that measures the ability of CB_2 receptors to mediate inhibition of lipopolysaccharide-induced release of tumour necrosis factor-α.
- Assays performed with cultured neurons that exploit the negative coupling of the CB_1 receptor to N- and P/Q-type calcium channels.
- Assays performed with isolated nerve-smooth muscle preparations, such as the mouse vas deferens, that exploit the ability of Δ^9-THC to act through neuronal CB_1 receptors to produce a concentration-related inhibition both of electrically-evoked contractile transmitter release and of the contractions resulting from this release.

Strategies for identifying CB_1 and/or CB_2 receptor-mediated effects, include the use of selective CB_1 and CB_2 receptor antagonists and control experiments with animals or tissues from which CB_1 and/or CB_2 receptors have been genetically deleted (reviewed in Howlett et al., 2002). In many bioassay systems commonly used cannabinoid receptor antagonists such as the CB_1-selective SR141716A, AM251 and AM281, and the CB_2-selective SR144528 and AM630, tend to produce effects opposite in direction from those produced by Δ^9-THC. The occurrence of these "inverse" effects presumably reflects the presence of background tone in these systems. Such tone may arise from ongoing release of endocannabinoids onto cannabinoid receptors. Alternatively, it may reflect spontaneous coupling of these receptors to their effector mechanisms, there being evidence that such coupling can occur and that all commercially available cannabinoid receptor antagonists can oppose this coupling by behaving as "inverse agonists" rather than "neutral" antagonists (reviewed in Pertwee, 2003b). Interestingly, two CB_1 receptor antagonists that do not produce an inverse effect and so behave as "neutral" antagonists, at least in the mouse isolated vas deferens, are the CBD analogue, (–)-6″-azidohex-2″-yne-CBD (O-2654) (Thomas et al., 2004), and a sulphonamide analogue of Δ^8-THC (O-2050) that, like O-2654, has an acetylenic side chain (Martin et al., 2002) (Figure 1).

There are no established bioassays for CBD. However this cannabinoid does produce several effects, albeit through pharmacological targets still to be identified, that are dose-related and could be exploited for the purpose of bioassay. Examples include its ability to induce signs of anxiolysis in animal models of anxiety, to prevent convulsions induced by electroshock or pentylenetetrazol and to produce apparent CB_1-independent surmountable antagonism of CB_1 receptor agonists in the mouse isolated vas deferens (Pertwee, 2004c; Pertwee et al., 2002; Thomas et al., 2004).

Therapeutic targets for Δ^9-THC and CBD

Δ^9-THC is already licensed for clinical use in the U.S.A. as an anti-emetic and appetite stimulant and both Δ^9-THC and cannabis extracts show therapeutic potential as neuroprotective and anticancer agents and for the management of glaucoma, pain and various kinds of motor dysfunction associated, for example, with multiple sclerosis and spinal cord injury (reviewed in Guzmán, 2003; Mechoulam et al., 2002; Pertwee, 2000a, 2000b, 2001, 2002, 2003a; Tomida et al., 2004). Particularly convincing are preclinical, anecdotal and clinical data supporting the use of cannabis extracts and Δ^9-THC against neuropathic pain and for the amelioration of spasticity, muscle spasms and pain associated with multiple sclerosis or spinal cord injury. Additional support for the use of cannabis or Δ^9-THC for multiple sclerosis was provided recently by results obtained in a

Table 6. Effects of cannabis and Δ^9-THC on signs and symptoms of multiple sclerosis in a double-blind, placebo-controlled multi-centre clinical trial[a]

Signs and symptoms	Treatment	Measured effect
611 male and female multiple sclerosis patients (aged 18 to 64), the primary outcome measure being the Ashworth score of spasticity	Cannabis[b,c] (p.o.) or Δ^9-THC[b,c] (p.o.) or Placebo[b]	No reduction in objective measure of spasticity (Ashworth Scale) Significant treatment effects: • symptoms of pain, spasms and spasticity ameliorated • quality of sleep improved • time to walk 10 m decreased (ambulant patients).
Also several secondary outcome measures.		Other notable findings: • high subjective placebo scores • similar results with cannabis & Δ^9-THC.

[a]Zajicek et al., 2003.
[b]Each patient received one treatment only – cannabis or Δ^9-THC or placebo.
[c]Target dose for 8 weeks was 5 to 12.5 mg Δ^9-THC twice daily.

large multi-centre clinical trial (Table 6). One problem that this trial encountered was the difficulty of measuring spasticity objectively, raising the possibility that perhaps spasticity experienced and assessed by the patients themselves rather than by an observer should form the basis of the primary outcome measure in future investigations. Consideration of the known effects of CBD (Table 4) suggests that it too has therapeutic potential, for example as an anti-inflammatory and neuroprotective agent, for the management of epilepsy, anxiety disorders, glaucoma and nausea, and for modulating some effects of Δ^9-THC or L-dihydroxyphenylalanine (reviewed in Pertwee, 2004c, 2004d). Such evidence has prompted the commercial development of Δ^9-THC- and CBD-rich cannabis extracts as medicines (Whittle et al., 2001).

Tolerance

Repeated administration of Δ^9-THC can cause tolerance to develop to a number of its effects and it is likely that this tolerance usually stems mainly from a reduction in the expression or density of cannabinoid receptors or in cannabinoid receptor signalling rather than from any change in the affinity of Δ^9-THC for these receptors or from some alteration in Δ^9-THC metabolism or pharmacokinetics (Maldonado, 2002; Pertwee, 1991, 1995, 1997). There is also evidence that cannabinoid CB_1 receptors are rapidly internalized following their activation by high-efficacy agonists (Hsieh et al., 1999; Keren & Sarne, 2003). However, it is likely that the process of CB_1 receptor internalization plays at most only a minor role in the production of tolerance by Δ^9-THC, probably because this cannabinoid has relatively low CB_1 receptor efficacy (Hsieh et al., 1999). Although reversible, tolerance to Δ^9-THC can persist for several weeks after drug withdrawal. Also, it seems to develop more readily and rapidly to some effects than to others. In mice, effects of Δ^9-THC to which tolerance develops particularly rapidly are hypothermia, hypokinesia and the elevation of plasma corticosterone levels (Pertwee, 1991). *In vivo* treatment of mice with Δ^9-THC rapidly renders vasa deferentia tolerant to Δ^9-THC-induced inhibition of electrically-evoked contractions when this is measured *in vitro* (Pertwee & Griffin, 1995; Pertwee et al., 1993). The same Δ^9-THC treatment causes these tissues to become tolerant to other cannabinoid receptor agonists but not to agonists for μ- δ- or κ-opioid receptors or for α_2-adrenoceptors, evidence that this tolerance is cannabinoid receptor agonist-specific. In contrast, cross-tolerance between Δ^9-THC and opioid receptor agonists has been detected for effects that are measured *in vivo* (Maldonado, 2002). The extent to which tolerance develops to clinically useful effects of Δ^9-THC remains to be established. In the meantime, there is preclinical evidence which suggests that at least some sought-after therapeutic effects of Δ^9-THC may be more resistant to tolerance development than some of its unwanted effects (De Vry et al., 2004). As to CBD, the extent to which tolerance can develop to this plant cannabinoid has been little investigated.

Future directions

Clearly, more is currently known about the pharmacological actions of Δ^9-THC than of CBD. However, even for Δ^9-THC, evidence is still emerging for the existence of additional pharmacological targets and it will now be important to establish the extent to which each of these proposed new targets contributes to the production of its sought-after medical effects and/or to the production of its unwanted effects. It is also clear that Δ^9-THC and CBD both have important potential as therapeutic agents and another major challenge must be to optimize their use in the clinic both separately and in combination by defining their therapeutic targets more precisely. Finally, Δ^9-THC and CBD are but two of many cannabis constituents and yet little is known about the pharmacology or therapeutic potential of these other constituents, alone or in combination, or about the extent to which these constituents can modulate the effects of Δ^9-THC and/or CBD. This too, should be the subject of future research.

Acknowledgments

This study was supported by grants from the National Institute on Drug Abuse (DA09789) and GW Pharmaceuticals.

References

Barann, M., G. Molderings, M. Brüss, H. Bönisch, B.W. Urban & M. Göthert, 2002. Direct inhibition by cannabinoids of human 5-HT_{3A} receptors: Probable involvement of an allosteric modulatory site. Br J Pharmacol 137: 589–596.

Bayewitch, M., M.-H. Rhee, T. Avidor-Reiss, A. Breuer, R. Mechoulam & Z. Vogel, 1996. (–)-Δ^9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. J Biol Chem 271: 9902–9905.

Cravatt, B.F. & A.H. Lichtman, 2002. The enzymatic inactivation of the fatty acid amide class of signaling lipids. Chem Phys Lipids 121: 135–148.

De Vry, J., K.R. Jentzsch, E. Kuhl & G. Eckel, 2004. Behavioral effects of cannabinoids show differential sensitivity to cannabinoid receptor blockade and tolerance development. Behav Pharm 15: 1–12.

Dinh, T.P., T.F. Freund & D. Piomelli, 2002. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121: 149–158.

Downer, E.J., M.P. Fogarty & V.A. Campbell, 2003. Tetrahydrocannabinol-induced neurotoxicity depends on CB_1 receptor-mediated c-Jun N-terminal kinase activation in cultured cortical neurons. Br J Pharmacol 140: 547–557.

Drysdale, A.J., R.G. Pertwee & B. Platt, 2004. Modulation of calcium homeostasis by cannabidiol in primary hippocampal culture. Br J Pharmacol (Proc Suppl) In press.

El-Remessy, A.B., I.E. Khalil, S. Matragoon, G. Abou-Mohamed, N.-J. Tsai, P. Roon, R.B. Caldwell, R.W. Caldwell, K. Green & G.I. Liou, 2003. Neuroprotective effect of (-)Δ^9-tetrahydrocannabinol and cannabidiol in *N*-methyl-D-aspartate-induced retinal neurotoxicity: Involvement of peroxynitrite. Am J Pathol 163: 1997–2008.

ElSohly, M.A., 2002. Chemical constituents of *cannabis*. In: F. Grotenhermen & E. Russo (Eds.), Cannabis and Cannabinoids. Pharmacology, Toxicology and Therapeutic Potential, pp. 27–36. Haworth Press, New York.

Fowler, C.J., 2003. Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents: non-psychoactive cannabinoids, 'entourage' compounds and inhibitors of *N*-acyl ethanolamine breakdown as therapeutic strategies to avoid psychotropic effects. Brain Res Rev 41: 26–43.

Gallily, R., T. Even-Chen, G. Katzavian, D. Lehmann, A. Dagan & R. Mechoulam, 2003. γ-Irradiation enhances apoptosis induced by cannabidiol, a non-psychotropic cannabinoid, in cultured HL-60 myeloblastic leukemia cells. Leuk Lymphoma 44: 1767–1773.

Grotenhermen, F., 2002. Effects of cannabis and cannabinoids. In: F. Grotenhermen & E. Russo (Eds.), Cannabis and Cannabinoids: Pharmacology Toxicology and Therapeutic Potential, pp. 55–65. Haworth Press, New York.

Guzmán, M., 2003. Cannabinoids: Potential anticancer agents. Nature Rev Cancer 3: 745–755.

Hampson, A.J., M. Grimaldi, J. Axelrod & D. Wink, 1998. Cannabidiol and (–)Δ^9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA 95: 8268–8273.

Hampson, A.J., M. Grimaldi, M. Lolic, D. Wink, R. Rosenthal & J. Axelrod, 2000. Neuroprotective antioxidants from marijuana. In: Reactive oxygen species: From radiation to molecular biology; Annals NY Acad Sci 899: 274–282.

Hanus, L., A. Breuer, S. Tchilibon, S. Shiloah, D. Goldenberg, M. Horowitz, R.G. Pertwee, R.A. Ross, R. Mechoulam & E. Fride, 1999. HU-308: A specific agonist for CB_2, a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 96: 14228–14233.

Herkenham, M., A.B. Lynn, M.R. Johnson, L.S. Melvin, B.R. de Costa & K.C. Rice, 1991. Characterization and localization of cannabinoid receptors in rat brain: A quantitative *in vitro* autoradiographic study. J Neurosci 11: 563–583.

Hillard, C.J. & A. Jarrahian, 2003. Cellular accumulation of anandamide: consensus and controversy. Br J Pharmacol 140: 802–808.

Hohmann, A.G., J.N. Farthing, A.M. Zvonok & A. Makriyannis, 2004. Selective activation of cannabinoid CB_2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. J Pharmacol Exp Ther 308: 446–453.

Howlett, A.C., F. Barth, T.I. Bonner, G. Cabral, P. Casellas, W.A. Devane, C.C. Felder, M. Herkenham, K. Mackie, B.R. Martin, R. Mechoulam & R.G. Pertwee, 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54: 161–202.

Hsieh, C., S. Brown, C. Derleth & K. Mackie, 1999. Internalization and recycling of the CB1 cannabinoid receptor. J Neurochem 73: 493–501.

Iversen, L.L., 2000. The Science of Marijuana. Oxford University Press, New York.

Iversen, L.L., 2003. Cannabis and the brain. Brain 126: 1252–1270.

Jordt, S.-E., D.M. Bautista, H. Chuang, D.D. McKemy, P.M. Zygmunt, E.D. Högestätt, I.D. Meng & D. Julius, 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427: 260–265.

Keren, O. & Y. Sarne, 2003. Multiple mechanisms of CB1 cannabinoid receptors regulation. Brain Res 980: 197–205.

Maldonado, R., 2002. Study of cannabinoid dependence in animals. Pharmacol Ther 95: 153–164.

Mamas, M.A. & D.A. Terrar, 1998. Differential sensitivity to cannabidiol of the two components of delayed rectifier potassium current in guinea-pig isolated ventricular myocytes. Br J Pharmacol 123: 319P.

Marsicano, G., B. Moosmann, H. Hermann, B. Lutz & C. Behl, 2002. Neuroprotective properties of cannabinoids against oxidative stress: Role of the cannabinoid receptor CB1. J Neurochem 80: 448–456.

Martin, B., L.A. Stevenson, R.G. Pertwee, C.S. Breivogel, W. Williams, A. Mahadevan & R.K. Razdan, 2002. Agonists and silent antagonists in a series of cannabinoid sulfonamides. Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p. 2.

Martin, B.R., B.F. Thomas & R.K. Razdan, 1995. Structural requirements for cannabinoid receptor probes. In: R.G. Pertwee (Ed.), Cannabinoid Receptors, pp. 35–85. Academic Press, London.

McKallip, R.J., C. Lombard, B.R. Martin, M. Nagarkatti & P.S. Nagarkatti, 2002. Δ^9-Tetrahydrocannabinol-induced apoptosis in the thymus and spleen as a mechanism of immunosuppression *in vitro* and *in vivo*. J Pharmacol Exp Ther 302: 451–465.

Mechoulam, R., D. Panikashvili & E. Shohami, 2002. Cannabinoids and brain injury: Therapeutic implications. Trends Mol Med 8: 58–61.

Molina-Holgado, E., C. Guaza, J. Borrell & F. Molina-Holgado, 1999. Effects of cannabinoids on the immune system and central nervous system: Therapeutic implications. Biodrugs 12: 317–326.

Murphy, L.L., 2002. Hormonal system and reproduction. In: F. Grotenhermen & E. Russo (Eds.), Cannabis and Cannabinoids: Pharmacology Toxicology and Therapeutic Potential, pp. 289–297. Haworth Press, New York.

Paton, W.D.M. & R.G. Pertwee, 1973a. The actions of cannabis in man. In R. Mechoulam (Ed.), Marijuana, pp. 287–333. Academic Press, New York.

Paton, W.D.M. & R.G. Pertwee, 1973b. The pharmacology of cannabis in animals. In: R. Mechoulam (Ed.), Marijuana, pp. 191–285. Academic Press, New York.

Pertwee, R.G., 1985. Effects of cannabinoids on thermoregulation: A brief review. In: D.J. Harvey (Ed.), Marihuana '84, pp. 263–277. IRL Press, Oxford.

Pertwee, R.G., 1988. The central neuropharmacology of psychotropic cannabinoids. Pharmacol Ther 36: 189–261.

Pertwee, R.G., 1991. Tolerance to and dependence on psychotropic cannabinoids. In: J.A. Pratt (Ed.), The Biological Bases of Drug Tolerance and Dependence, pp. 231–263. Academic Press, London.

Pertwee, R.G., 1995. Pharmacological, physiological and clinical implications of the discovery of cannabinoid receptors: An overview. In R.G. Pertwee (Ed.), Cannabinoid Receptors, pp. 1–34. Academic Press, London.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol Ther 74: 129–180.

Pertwee, R.G., 2000a. Cannabinoid receptor ligands: Clinical and neuropharmacological considerations relevant to future drug discovery and development. Exp Opin Invest Drugs 9: 1553–1571.

Pertwee, R.G., 2000b. Neuropharmacology and therapeutic potential of cannabinoids. Addict Biol 5: 37–46.

Pertwee, R.G., 2001. Cannabinoid receptors and pain. Prog Neurobiol 63: 569–611.

Pertwee, R.G., 2002. Cannabinoids and multiple sclerosis. Pharmacol Ther 95: 165–174.

Pertwee, R.G., 2003a. Cannabinoids. In: C. Bountra, R. Munglani & W.K. Schmidt (Eds.), Current Understanding, Emerging Therapies, and Novel Approaches to Drug Discovery, pp. 683–706. Marcel Dekker, New York.

Pertwee, R.G., 2003b. Inverse agonism at cannabinoid receptors. In: A.P. IJzerman (Ed.), Inverse Agonism, pp. 75–86. Elsevier, Amsterdam.

Pertwee, R.G., 2004a. Novel pharmacological targets for cannabinoids. Curr Neuropharmacol 2: 9–29.

Pertwee, R.G., 2004b. Pharmacological actions of cannabinoids. In: R.G. Pertwee (Ed.), Cannabinoids. Handbook of Experimental Pharmacology. Springer-Verlag, Berlin. In press.

Pertwee, R.G., 2004c. The pharmacology and therapeutic potential of cannabidiol. In: V. Di Marzo (Ed.), Cannabinoids, Kluwer Academic/Plenum Publishers, pp. 32–83.

Pertwee, R.G., 2004d. The therapeutic potential of cannabidiol. In: R. Mechoulam (Ed.), Cannabinoids as Therapeutics, Birkhauser Publishing, Basle, In press.

Pertwee, R.G. & G. Griffin, 1995. A preliminary investigation of the mechanisms underlying cannabinoid tolerance in the mouse vas deferens. Eur J Pharmacol 272: 67–72.

Pertwee, R.G., R.A. Ross, S.J. Craib & A. Thomas, 2002. (–)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. Eur J Pharmacol 456: 99–106.

Pertwee, R.G., L.A. Stevenson & G. Griffin, 1993. Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212–2 and anandamide. Br J Pharmacol 110: 1483–1490.

Platt, B. & A.J. Drysdale, 2004. Search and rescue: Identification of cannabinoid actions relevant for neuronal survival and protection. Curr Neuropharmacol 2: 103–114.

Poling, J.S., M.A. Rogawski, N. Salem & S. Vicini, 1996. Anandamide, an endogenous cannabinoid, inhibits *Shaker*-related voltage-gated K^+ channels. Neuropharmacology 35: 983–991.

Quartilho, A., H.P. Mata, M.M. Ibrahim, T.W. Vanderah, F. Porreca, A. Makriyannis & T.P. Malan, 2003. Inhibition of inflammatory hyperalgesia by activation of peripheral CB_2 cannabinoid receptors. Anesthesiology 99: 955–960.

Thomas, A., R.A. Ross, B. Saha, A. Mahadevan, R.K. Razdan & R.G. Pertwee, 2004. 6″-Azidohex-2″-yne-cannabidiol: A potential neutral, competitive cannabinoid CB_1 receptor antagonist. Eur J Pharmacol 487: 213–221.

Tomida, I., R.G. Pertwee & A. Azuara-Blanco, 2004. Cannabinoids and glaucoma. Br Ophthalmol 88: 708–713.

Ueda, N., 2002. Endocannabinoid hydrolases. Prostaglandins Other Lipid Mediat 68–9: 521–534.

van der Stelt, M. & V. Di Marzo, 2004. Metabolic fate of endocannabinoids. Curr Neuropharmacol 2: 37–48.

van der Stelt, M., W.B. Veldhuis, M. Maccarrone, P.R. Bär, K. Nicolay, G.A. Veldink, V. Di Marzo & J.F.G. Vliegenthart, 2002.

Acute neuronal injury, excitotoxicity, and the endocannabinoid system. Mol Neurobiol 26: 317–346.

Whittle, B.A., G.W. Guy & P. Robson, 2001. Prospects for new cannabis-based prescription medicines. J Cannabis Ther 1: 183–205.

Zajicek, J., P. Fox, H. Sanders, D. Wright, J. Vickery, A. Nunn & A. Thompson, 2003. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial. Lancet 362: 1517–1526.

Zygmunt, P.M., D.A. Andersson & E.D. Högestätt, 2002. Δ^9-tetrahydrocannabinol and cannabinol activate capsaicin-sensitive sensory nerves via a CB_1 and CB_2 cannabinoid receptor-independent mechanism. J Neurosci 22: 4720–4727.