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Cannabis and endogenous cannabinoid systems

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1. Introduction

A series of remarkable developments during the last 10 years have provided conclusive evidence that Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent in marijuana, binds to specific cannabinoid receptors and mimics the actions of endogenous cannabinoids. The significance of the endogenous cannabinoid system is underscored by the finding that cannabinoid receptors exist in the brain at levels higher than most other G protein-coupled receptors, approaching levels observed for amino acid receptors. In many ways, the development of this field parallels that of the opioid receptor field almost 20 years earlier: both fields derive from plant products used for thousands of years; the receptors for both drug systems belong to the superfamily of G protein-coupled receptors; and both systems contain endogenous compounds which exert important biological effects of their own. However, the different structures of opioid and cannabinoid receptors, and the different distributions of these receptors in the brain, mean that these two drug systems exert very different spectra of effects, and represent different potentials for drug abuse. Most important, while the therapeutic benefits of morphine as an analgesic have been established for centuries and are still widely used today, the therapeutic utility for Δ^9-THC in a variety of conditions is a widely debated issue.

The purpose of this article is to review the principal aspects of the endogenous cannabinoid systems in the brain. More extensive recent reviews on specific topics of cannabinoid actions are available (Howlett, 1995; Abood and Martin, 1996; Adams and Martin, 1996; Childers and Deadwyler, 1996; Onaivi et al., 1996; Pertwee, 1997; Breivogel and Childers, 1998).

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2. History and effects of cannabis use

Cannabis sativa (Latin for 'planted hemp') grows as both male and female plants. While cannabis growing in hotter, dryer climates produces more of the resin containing the psychoactive ingredients, cannabis grown in more temperate zones produces a more fibrous stalk. There has been debate as to whether separate species of cannabis exist, or whether these divergent characteristics are merely a result of environmental influences (Abel, 1980).

Cannabis may be the earliest non-food-bearing plant cultivated by humans. The earliest archaeological evidence of cannabis use dates back 10 000 years (Fig. 1), when fibers of the stalk were used in Stone Age Taiwan. The earliest recorded use of cannabis is approx. 2700 BC when the 'father of Chinese medicine' described the use of cannabis to treat several conditions. In ancient India, cannabis use played a role in religion, as described in the Vedas written around 2000 BC. Hashish, prepared from the sticky resin of cannabis, was known in the Arab world since the 10th century AD (Abel, 1980).

In the mid-19th century, Western medicine took notice of the potential medical uses of cannabis due to the research of Dr William Brooke O'Shaughnessy; however, medical marijuana research was terminated in the US in 1906. The US Congress effectively outlawed marijuana in 1937 with the Marijuana Tax Act. According to the National Household Survey on Drug Abuse, 4–5% of the general population and 15–20% of high school seniors and college students used marijuana at least once a month in 1994. Americans spent an estimated $14 billion on 2000 metric tons of marijuana each year from 1990 to 1993 (Chalsma and Boyum, 1994).

The primary psychoactive constituents of cannabis, Δ^8-THC and Δ^9-THC, were isolated in 1964 (Gaoni and Mechoulam, 1964). Cannabis contains other related compounds, but only Δ^8-THC and Δ^9-THC ex-

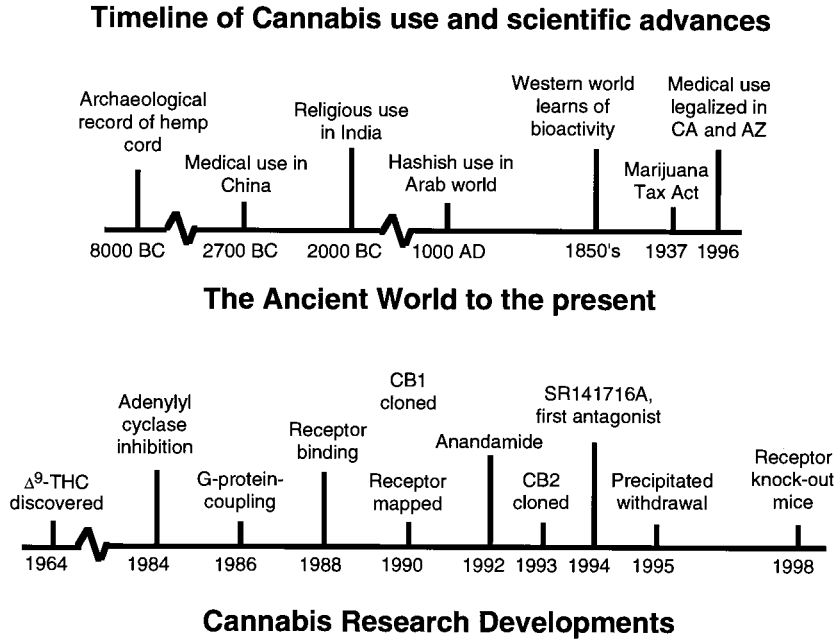

Fig. 1. Timeline. A brief history of records of cannabis use throughout the world and advances in research on the mechanisms of cannabinoid actions.

hibit all of the psychoactive effects of cannabis. Δ^9-THC is more prevalent in marijuana and more potent in vivo than Δ^8-THC, and thus most of the psychoactivity has been attributed to Δ^9-THC (Pertwee, 1988). The primary product of metabolic transformation of Δ^9-THC by the liver, 11-OH-Δ^9-THC, is actually more potent than Δ^9-THC (Howlett, 1995). Moreover, there is a considerable lag time between peak plasma concentrations of Δ^9-THC and the 'high' reported by human subjects (Hollister et al., 1981), implicating a metabolite as a major biologically active compound.

The effects of cannabis and Δ^9-THC in humans include: disruption of short-term memory, cognitive impairments, a sense of time dilation, enhanced body awareness, discoordination, sleepiness, dry mouth, conjunctival injection (red eyes), mood alterations, tachycardia and immune modulation (Hollister, 1986). Mood alterations may include euphoria or dysphoria, and depends on prior experience of the user, mood state at the time of onset, drug dose and route of administration (Pertwee, 1988). Dysphoric effects occur more frequently with oral as opposed to smoked administration, since it is easier to titrate the dose by smoking. Some effects of cannabinoids may be therapeutically useful, including antiemetic, analgesic, antispasmodic, appetite-stimulating and sleep-inducing effects. Dronabinol (Marinol®, or synthetic Δ^9-THC) is the only cannabinoid used medically, and is given orally as an antiemetic and appetite-stimulant for cancer chemotherapy patients. The Institute of Medicine is currently (1998) conducting risk/benefit analysis of marijuana for a variety of therapeutic conditions.

3. Cannabinoid receptors and endogenous cannabinoids

3.1. Development of synthetic cannabinoids

Δ^9-THC is relatively weak in binding to brain cannabinoid receptors, and is not a full agonist at the level of the transducer (Sim et al., 1996a). Therefore early cannabinoid research was hampered by the lack of potent cannabinoid agonists and antagonists. Progress in identifying cannabinoid receptors came from the development of potent agonists, which to date belong to four major categories (Fig. 2). Δ^9-THC and its metabolites belong to the classical cannabinoid group, a series of dibenzopyran derivatives. More potent derivatives, including CP55940, were developed by Pfizer as bicylic and tricyclic analogs of Δ^9-THC lacking the pyran ring (Johnson and Melvin, 1986). CP55940 was the first ^{3}H-labeled cannabinoid with sufficient potency and selectivity for receptor binding to both brain membranes (Devane et al., 1988) and brain sections (Herkenham et al., 1990, 1991b). The third group of compounds, represented by the potent agonist WIN 55212-2, are the aminoalkylindoles. These analogs were originally synthesized by Sterling Winthrop as cyclooxygenase inhibitors. The discovery that these compounds inhibited electrically-stimulated contractions of mouse vas deferens and adenylyl cyclase in brain membranes (Ward et al., 1990; Pacheco et al., 1991) led to the concept that they acted through cannabinoid receptors. The fourth series of cannabinoid agonists are arachidonic

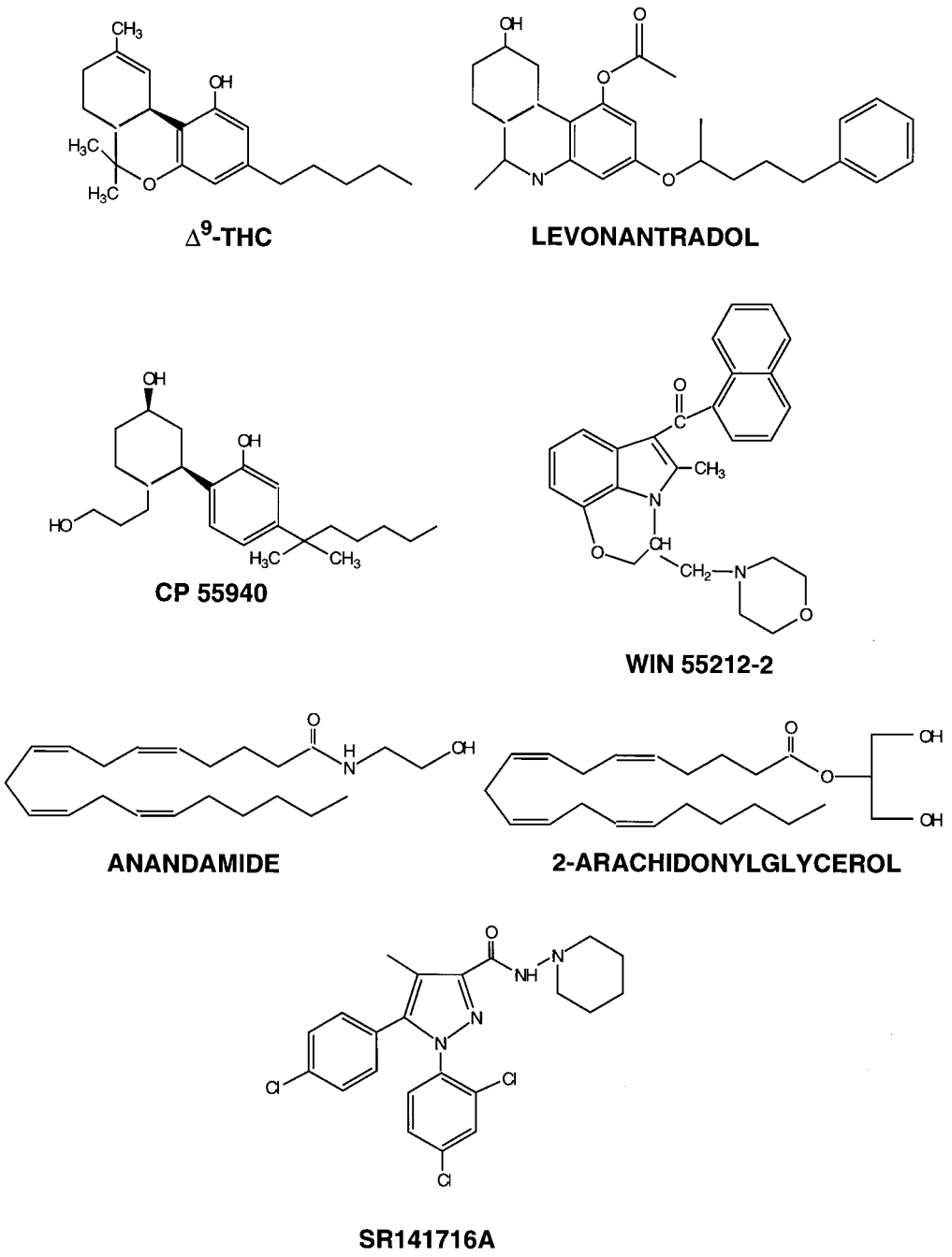

Fig. 2. Chemical structures of commonly used cannabinoids.

acid derivatives, typified by anandamide (arachidonyl ethanolamide), the first endogenous cannabinoid isolated from mammalian brain (Devane et al., 1992).

Another major factor which impeded cannabinoid research was the lack of a pharmacologically specific antagonist. Evidence that certain aminoalkylindole analogs, including WIN 56,098 and WIN 54,461, acted as cannabinoid antagonists in vitro was inconclusive, since the potencies of these analogs were too low for practical use. The first specific cannabinoid antagonist was SR141716A (Fig. 2), synthesized by Sanofi (Rinaldi-Carmona et al., 1994). This compound is a pure antagonist at low (nM) concentrations, has both high potency and selectivity for CB1 vs. CB2 receptors, and blocks the actions of various cannabinoid agonists in vivo (Compton et al., 1996). Another recently reported CB1 antagonist is AM630 (Hosohata et al., 1997), while Sanofi has recently reported the development of a CB2-selective antagonist, SR144528 (Barth et al., 1997). Other results have suggested that Δ^9-THC itself is an antagonist at CB2 receptors (Bayewitch et al., 1996), which is consistent with the

low efficacy of Δ^9-THC for activating G proteins through brain cannabinoid receptors (Sim et al., 1996a). More recently, an eicosanoid derivative, methyl arachidonyl fluorophosphonate, has been reported as an irreversible CB1 antagonist (Fernando and Pertwee, 1997).

3.2. Discovery of cannabinoid receptors

Although several studies suggested that Δ^9-THC actions were mediated by changes in membrane fluidity (Hillard et al., 1985), a wealth of pharmacological data indicated a specific structure–activity relationship for cannabinoids, implying a receptor-mediated mechanism (Dewey, 1986). The existence of cannabinoid receptors was confirmed when Howlett showed that cannabinoids decreased cAMP in neuroblastoma cell cultures (Howlett, 1984), suggesting mediation by a $G_{i/o}$-coupled receptor (Howlett and Fleming, 1984; Howlett, 1985; Howlett et al., 1986). This finding was followed by cannabinoid receptor radioligand binding (Devane et al., 1988), receptor localization (Herkenham et al., 1990), cloning and sequencing of the brain cannabinoid receptor CB1 (Matsuda et al., 1990), and the discovery of CB1A, an alternative splice variant of CB1 (Shire et al., 1995). The cloning of a peripheral cannabinoid receptor, CB2, from spleen cells (Munro et al., 1993) showed that there are at least two major types of cannabinoid receptors. Both receptor types are members of the seven transmembrane-domain, G protein-coupled family of receptors, with 44% homology between CB1 and CB2 (Fig. 3). CB1 is larger than CB2, with an additional 72 amino acid residues in the N-terminal region, 15 additional residues in the third extracellular loop, and 13 additional residues in the C-terminal region. The highest degree of homology between CB1 and CB2 occurs in the transmembrane regions TM2, TM3, TM5 and TM6; interestingly, the homology in other regions is not particularly striking. This lack of homology suggests that the design of selective agonists and antagonists to distinguish between these two receptor types should be possible, but this area of pharmacology is still in the early stages of development.

Since the discovery of CB1 and CB2 receptors, and the development of receptor binding assays in both brain membranes (Devane et al., 1988) and transfected cell lines (Felder et al., 1995; Showalter et al., 1996), a number of structure–activity studies of synthetic cannabinoid ligands have been reported (Compton et al., 1993; Adams et al., 1995; Reggio et al., 1997). These data have been used to develop models of cannabinoid binding sites (Thomas et al., 1991; Bramblett et al., 1995; Xie et al., 1996).

3.3. Endogenous cannabinoids

Using the logic that an endogenous cannabinoid substance would exhibit the same lipophilic properties of known cannabinoids, Devane et al. (1992) used chloroform/methanol extracts of porcine brain to

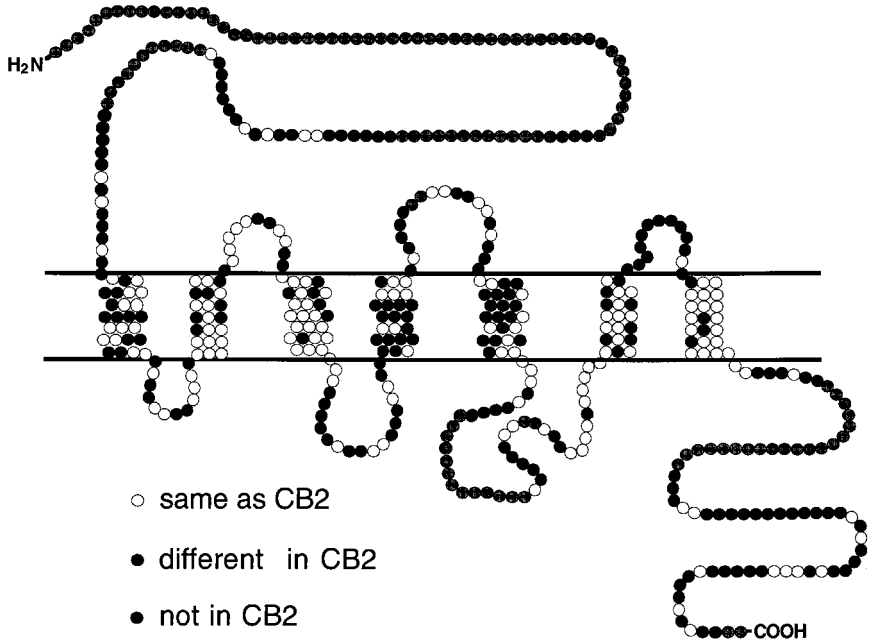

Fig. 3. Schematic of the primary structures of CB1 and CB2 receptors. The horizontal lines represent the borders of the plasma membrane with the receptor oriented with the extracellular domain at the top. The structure shown is that of CB1; amino acids that are identical in CB2 are shown as open circles, those that are different are shown as filled circles, and those that are not present in CB2 are shown as shaded circles.

isolate endogenous compounds that inhibited [^{3}H]CP55940 binding to brain membranes. The first such substance, arachidonyl ethanolamine, was named anandamide (Fig. 2) derived from the Sanskrit word for 'bliss'. Anandamide exhibits a moderate affinity for CB1 receptors (approx. 100 nM), and is extremely unstable, since it is rapidly metabolized by amidases (Deutsch and Chin, 1993). This metabolism can be prevented by the use of amidase inhibitors like phenylmethylsulfonylfluoride (PMSF) (Deutsch and Chin, 1993; Childers et al., 1994), or can be avoided with metabolically stable forms of anandamide, such as methanandamide and others (Abadji et al., 1994). Nevertheless, the instability of anandamide creates special problems in interpreting a number of experiments in this field, and may explain in vivo studies where anandamide failed to demonstrate full cannabinoid-like activity (Wiley et al., 1995). Anandamide is also a partial agonist at CB1 receptors, as determined by its ability to activate G proteins (Breivogel et al., 1998), inhibit adenylyl cyclase (Childers et al., 1994) and affect ion channel function (Mackie et al., 1993; Shen et al., 1996). The biological significance of this phenomenon is unknown, but it represents one of the few examples in which an endogenous neurotransmitter/neuromodulator is a partial agonist at its receptor.

Anandamide has both central and peripheral effects. In the periphery, anandamide has been implicated as an endothelium-derived hyperpolarizing factor (Randall et al., 1996), although this role has been questioned (White and Hiley, 1997). In the brain, the identity of anandamide as a potential neurotransmitter has only partially been established. For example, its precise neuroanatomical localization is not yet known because specific neuroanatomical methodologies are not yet available. Nevertheless, several other aspects of its function have been extensively examined. The first studies of anandamide synthesis focused on the condensation of ethanolamine and arachidonic acid by anandamide synthase (Deutsch and Chin, 1993). It is now clear that this mechanism is not the physiologically relevant method of anandamide synthesis, and instead simply represents the reverse reaction of the hydrolytic enzyme anandamide amidase (Arreaza et al., 1997). Other studies focused on an alternative synthetic pathway in which *N*-arachidonyl phosphatidyl ethanolamine (NAPE) is cleaved by phospholipase D to yield phosphatidic acid and anandamide (Cadas et al., 1997; Sasaki and Chang, 1997). This reaction occurs in the brain, and is regulated by cAMP in a way which would permit negative feedback regulation of anandamide synthesis by anandamide binding to cannabinoid receptors to inhibit adenylyl cyclase (Cadas et al., 1996).

Two mechanisms for the inactivation of anandamide have been identified in the brain. The first is the amidase which cleaves anandamide to arachidonic acid and ethanolamine, as mentioned above. This enzyme has been extensively characterized and cloned (Cravatt et al., 1996). In addition to PMSF, several eicosanoid inhibitors of this enzyme have been synthesized (Deutsch et al., 1997a,b). A second major form of anandamide inactivation is presynaptic uptake. As originally described by Piomelli and colleagues, anandamide transport in neurons exhibits many of the same properties of other neurotransmitter transport systems, and one compound, AM404, has been identified as a potentially selective inhibitor of anandamide transport (Beltramo et al., 1997). An important question is whether this transport system or the amidase enzyme is primarily responsible for anandamide inactivation. It is possible that both systems act in concert, with the uptake systems acting to transport anandamide into neurons after its release, followed by the intracellular hydrolysis of anandamide.

Several other eicosanoid derivatives that bind to cannabinoid receptors have been isolated from different tissues. One compound of potential significance is 2-arachidonyl glycerol (Fig. 2), originally isolated from dog intestine (Mechoulam et al., 1995) and rat brain (Sugiura et al., 1995). Although it exhibits a lower affinity for CB1 than anandamide, evidence suggests that it is present in the brain at higher levels than anandamide; moreover, it is a full agonist at CB1 receptors, and it inhibits long-term potentiation in hippocampal slice electrophysiological experiments (Stella et al., 1997). All these experiments support the concept that 2-arachidonyl glycerol is a primary endogenous cannabinoid in the brain.

Endogenous cannabinoids may also be non-eicosanoid in nature. Howlett and colleagues (Evans et al., 1994) isolated material from brain extracts which inhibits [^{3}H]CP55940 binding and is released from brain slices in a Ca^{2+}-dependent manner. Although its structure is not yet known, it appears to be a peptide. Similarly, our own laboratory has isolated a hydrophilic compound from bovine brain which inhibits both [^{3}H]WIN 55212-2 and [^{3}H]SR141716A binding to cerebellar membranes (Deadwyler et al., 1995a). Although its structure is also not yet known, its behavior on several chromatographic systems suggests that it is not related chemically to the eicosanoids.

4. Signal transduction mechanisms of cannabinoid receptors

4.1. G protein-coupling

As observed with other G protein-coupled recep-

tors, guanine nucleotides inhibit cannabinoid agonist binding (Devane et al., 1988). Moreover, cannabinoid binding sites can be solubilized together with G proteins from membranes (Houston and Howlett, 1993). Cannabinoid receptor activation of G proteins in isolated membranes can be measured by agonist-stimulated [^{35}S]guanylyl-5′-*O*-(γ-thio)-triphosphate ([^{35}S]GTPγS) binding (Selley et al., 1996), which follows the pharmacology of the brain cannabinoid receptor binding profile. In rat brain membranes, cannabinoid-stimulated [^{35}S]GTPγS binding is especially high because of the relatively large number of cannabinoid receptors in the brain (Kuster et al., 1993). However, cannabinoid receptor coupling to G proteins is relatively inefficient: in striatum, each cannabinoid receptor activates only three G proteins, compared to 20 G proteins for each mu (μ) and delta (δ) opioid receptor (Sim et al., 1996b; Breivogel et al., 1997). It is possible that this relatively low amplification is related to the high number of CB1 receptors: since these receptors exist in such high number in the brain, a high amplification between the receptor and transducer may not be necessary.

Agonist-stimulated [^{35}S]GTPγS binding can also be used to determine differences in agonist efficacy at the level of the G protein (Sim et al., 1996a; Selley et al., 1997). Using this technique in brain membranes, WIN 55212-2 and levonantradol are full agonists, while anandamide produces partial efficacy (Breivogel et al., 1998), and Δ^9-THC is a weak partial agonist (Sim et al., 1996a; Burkey et al., 1997). There is a discrepancy in the actions of the CB1 antagonist SR141716A in [^{35}S]GTPγS experiments: in rat cerebellar membranes, SR141716A is a neutral antagonist, with no effect on [^{35}S]GTPγS binding except at concentrations 10 000 times greater than its affinity at CB1 receptors (Breivogel et al., 1998), while in CB1-transfected CHO (Chinese hamster ovary) cells, SR141716A is an inverse agonist, producing relatively potent inhibition of basal [^{35}S]GTPγS binding (Landsman et al., 1997).

Are the classical behavioral patterns elicited by Δ^9-THC correlated with the G protein-coupled cannabinoid receptor binding site? When the affinities of cannabinoids in binding to brain receptors are compared to in vivo potencies in eliciting these behavioral patterns in rats, highly significant correlations have been observed. These correlations have been obtained for traditional cannabinoids and their analogs (Thomas et al., 1991; Compton et al., 1992, 1993; Melvin et al., 1993), and for a series of anandamide analogs as well (Adams et al., 1995; Thomas et al., 1996). The conclusion from all of these studies is that there is no reason to suggest that non-receptor-mediated mechanisms are responsible for the behavioral effects of Δ^9-THC observed in rats. Whether such a correlation is true for humans, with psychoactivity as a behavioral end-point, is unknown.

4.2. Cannabinoid receptor coupled effectors

Cannabinoid receptor activation of G proteins influences multiple effector systems (Fig. 4). Cannabinoid inhibition of adenylyl cyclase has been demonstrated in several cell types (Howlett, 1984; Pacheco et al., 1993; Bayewitch et al., 1995; Felder et al., 1995; Slipetz et al., 1995), and in brain membranes (Bidaut-Russell et al., 1990; Pacheco et al., 1991, 1994; Childers et al., 1994). In general terms, the pharmacology of cannabinoid-inhibited adenylyl cyclase matches that of cannabinoid receptor binding (Pacheco et al., 1991), including competitive antagonism by SR141716A (Rinaldi-Carmona et al., 1994; Felder et al., 1995). The finding (Pacheco et al., 1993) that responses of several $G_{i/o}$-coupled receptor agonists are non-additive with cannabinoids in inhibiting adenylyl cyclase, but additive at the level of G proteins, suggests that cannabinoid receptors share common adenylyl cyclase catalytic units but different G proteins with other receptors like adenosine A_1 and $GABA_B$ receptors. In addition to inhibiting adenylyl cyclase, cannabinoids have been shown to stimulate cAMP accumulation in both globus pallidus slices (Maneuf and Brotchie, 1997) and striatal neurons (Glass and Felder, 1997), possibly via activation of G_s.

As with other receptors coupled to $G_{i/o}$ proteins, activation of CB1 receptors decreases Ca^{2+} conductance (Caulfield and Brown, 1992; Mackie and Hille, 1992; Mackie et al., 1993; Shen et al., 1996) and increases K^+ conductance (Mackie et al., 1995). These effects on Ca^{2+} channels (Mackie and Hille, 1992) and G protein-coupled inwardly-rectifying K^+ (GIRK) channels (Henry and Chavkin, 1995) are due to direct coupling by G proteins and independent of cAMP (Fig. 4). There may be important differences in CB1- and CB2-mediated effectors, since CB2 receptors in transfected CHO cells had no effect on these ion currents despite their ability to inhibit adenylyl cyclase (Felder et al., 1995).

In addition to ion channels, other G protein-coupled effectors have been associated with cannabinoid receptors; the most well known is the cannabinoid-induced release of arachidonic acid, observed in several cell culture systems and mediated both by phospholipase activity and G proteins (Audette et al., 1991; Burstein et al., 1994; Shivachar et al., 1996). Cannabinoids acting via both CB1 (Bouaboula et al., 1995) and CB2 (Bouaboula et al., 1996) receptors activate mitogen-activated protein (MAP) kinase and induce Krox-24 expression, presumably via activation of G protein $\beta\gamma$ subunits. These findings illustrate the basic concept that G protein-coupled receptors may

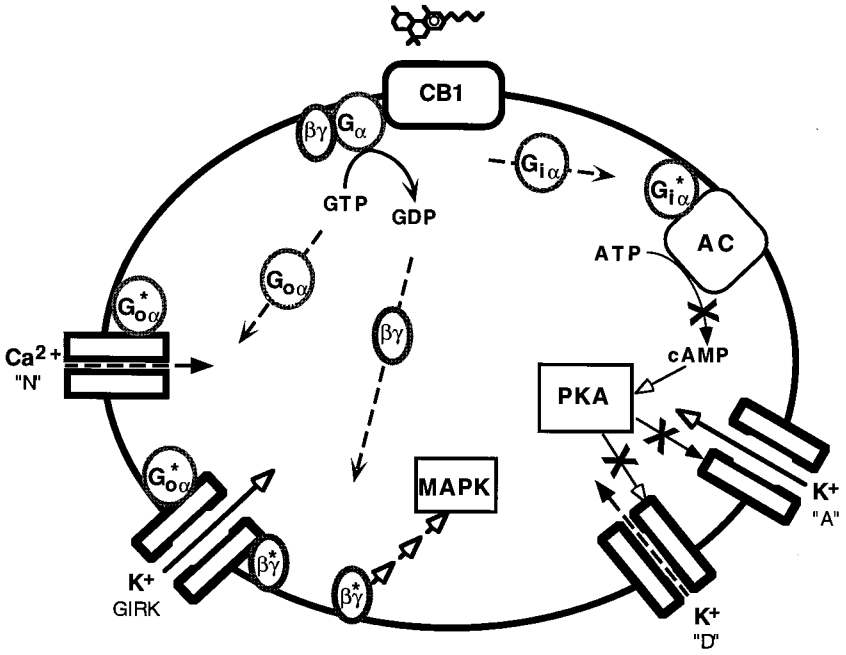

Fig. 4. Several proposed signal transduction mechanisms of cannabinoid receptors. After binding by agonist (top), CB1 activates G proteins ($G_{i\alpha}$, $G_{o\alpha}$ and $\beta\gamma$), which in turn act upon various effectors including: adenylyl cyclase (AC), Ca^{2+} and K^+ channels, and mitogen-activated protein kinase (MAPK). Inhibition of AC and subsequent decreases in cAMP decreases activation of cAMP-dependant protein kinase (PKA), which leads to decreased phosphorylation of the K^+ channels shown. Stimulatory effects are shown by open arrows and inhibitory effects by filled arrows. The 'open' or 'closed' states of the channels and X's over some arrows reflect the final effect of cannabinoid agonists.

operate through several different effectors, and each effector system may be designed for specific purposes.

Cannabinoids also alter conductance through potassium channels responsible for 'A' current in primary cultures of hippocampal neurons (Deadwyler et al., 1993). Cannabinoid agonists produce a positive shift in the voltage dependence of this channel; this process is cAMP-dependent and is mediated by cannabinoid inhibition of adenylyl cyclase and subsequent inhibition of protein phosphorylation (Childers et al., 1993; Deadwyler et al., 1995b; Hampson et al., 1995). Interestingly, addition of the CB1 antagonist SR141716A alone produced effects on 'A' current similar to elevating cAMP (Mu et al., 1995), suggesting either a population of constitutively active receptors, or a resting level of endogenous ligand. Cannabinoids have recently been shown to decrease K^+ 'D' currents in an manner opposite to the regulation of the 'A' current, since phosphorylation of 'A' and 'D' channels has opposing effects on the respective K^+ currents (Fig. 4). Thus cannabinoids alter the basal K^+ current profile from a mixture of 'A' and 'D' to a pure 'A' current (S.A. Deadwyler, personal communication).

5. Functional neuroanatomy of cannabinoids

5.1. Distribution of cannabinoid receptors

The highest levels of CB1 receptors are found in the CNS, but CB1 is also found in testis, uterus, spleen, heart, vas deferens and urinary bladder of various rodents (Pertwee, 1997). CB2 receptors are generally not found in the central nervous system (Munro et al., 1993), but there is a report implicating CB2 in mouse cerebellum (Skaper et al., 1996). CB2 receptors are found primarily on cells of the immune system (especially B cells), and have been implicated in the immunomodulating effects of cannabinoids (Kaminski et al., 1992).

Although cannabinoid receptors are distributed throughout the mammalian CNS (Herkenham et al., 1990, 1991b; Jansen et al., 1992; Mailleux and Vanderhaeghen, 1992; Glass et al., 1997), the regional distribution of cannabinoid receptors and cannabinoid-receptor activated G proteins (Fig. 5) correspond well with the behavioral effects of cannabinoids. Cannabinoid receptor density is highest in the outflow nuclei of the basal ganglia, including substantia nigra pars reticulata, entopeduncular nucleus and globus pallidus. High levels of these receptors are also found in the putamen and the molecular layers of the cerebellum and hippocampal dentate gyrus (Herkenham et al., 1991b). It must be noted that cannabinoid receptor density is extremely high compared to other G protein-coupled receptors in the brain, rivalling levels of amino acid-gated ion channels (Herkenham et al., 1990). Thus, even areas that exhibit relatively low levels of cannabinoid receptors may have rela-

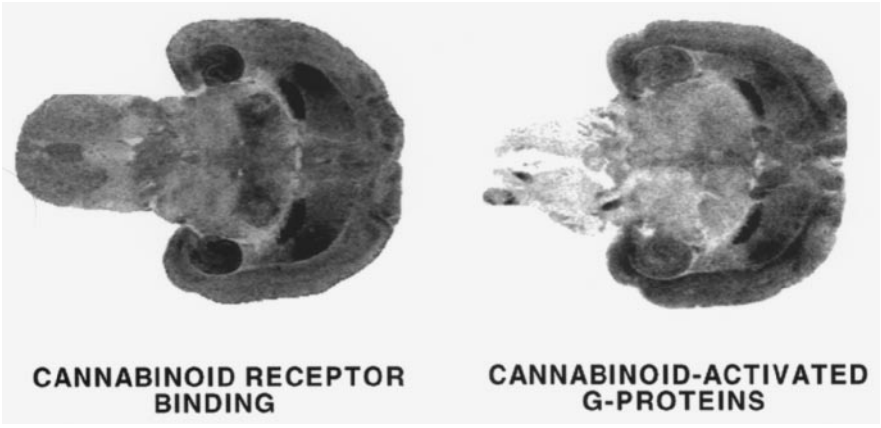

Fig. 5. In vitro autoradiography of cannabinoid receptor binding and cannabinoid receptor-activated G proteins in horizontal sections of rat brain. Cannabinoid receptor distribution is shown at the left as [^{3}H]WIN 55212-2 binding, and the distribution of cannabinoid-activated G proteins is shown at the right as WIN 55212-2-stimulated [^{35}S]GTPγS binding. Areas shown with highest receptor labeling include hippocampus, globus pallidus, striatum and inner layers of cortex.

tively high levels compared to other G protein-coupled receptors.

Brain localization of cannabinoid-activated G proteins, as determined with [^{35}S]GTPγS autoradiography (Sim et al., 1995), shows the same regional distribution as cannabinoid receptor binding (Breivogel et al., 1997). However, regional differences in the ratio of cannabinoid-activated G proteins to cannabinoid receptors are apparent (Breivogel et al., 1997). This indicates that the amount of cannabinoid activity in a brain region cannot be predicted solely based on relative receptor density.

5.2. Motor effects

The effects of cannabinoids on movement are readily apparent in rodents, and appear to be biphasic, with low doses stimulating and higher doses inhibiting locomotion. Since the majority of the effects of these compounds on neuronal activity are inhibitory, the neuronal substrate of the inhibitory locomotor effects have been attributed to the high density of cannabinoid receptors in the basal ganglia and cerebellum. Cannabinoid receptors in the outflow nuclei of the basal ganglia are localized to the axon terminals of neurons projecting from the striatum and subthalamic nucleus (Herkenham et al., 1991a; Mailleux and Vanderhaeghen, 1992). Thus, it appears that cannabinoids regulate both inhibitory and excitatory inputs to the basal ganglia, since striatal projections to these areas are GABAergic and subthalamic projections are glutamatergic (Alexander and Crutcher, 1990). Cannabinoid receptors in striatum may be pre-synaptic on striatal neurons and post-synaptic from corticostriate projections (Herkenham et al., 1991a; Mailleux and Vanderhaeghen, 1992).

In the substantia nigra, cannabinoids decrease transmission both from the striatum and subthalamic nucleus (Sañudo-Peña and Walker, 1997) through presynaptic cannabinoid receptors. Thus, cannabinoid receptors decrease both the inhibitory and stimulatory inputs to the substantia nigra, and may provide dual regulation of movement at this nucleus. Similar studies have been conducted in the globus pallidus, which has been implicated in mediating the cataleptic effects of cannabinoids (Pertwee and Wickens, 1991). Unlike the effect in substantia nigra (Miller and Walker, 1996), cannabinoids either increased or decreased globus pallidus neuronal activity, depending on whether the striatal projection neurons were stimulated. Furthermore, the reuptake, but not release of GABA was blocked by cannabinoids (Maneuf et al., 1996a,b). Other possible sources of cannabinoid effects on movement are receptors in the cortex (Herkenham et al., 1991b; Glass et al., 1997), stimulatory corticostriate projections (Mailleux and Vanderhaeghen, 1992) and cerebellar cannabinoid receptors.

5.3. Memory effects

One of the primary effects of marijuana in humans is disruption of memory (Hollister, 1986), consistent with high levels of CB1 located in hippocampus. This effect has been modeled in animals using delayed-match-to-sample behavioral assessment, where the effects of Δ^9-THC resemble a hippocampal lesion (Heyser et al., 1993). Cannabinoid receptors in the dentate gyrus may be localized to neurons of the hilus and on axon terminals of the cholinergic projections from the septum and the diagonal band of Broca. Cannabinoid receptors in CA1 and CA3 are likely to be localized to both pyramidal neurons and interneu-

rons, and located on the projections from septum and the diagonal band of Broca (Mailleux and Vanderhaeghen, 1992).

Deadwyler and colleagues have demonstrated that cannabinoids decrease neuronal activity in the hippocampus and its inputs (Campbell et al., 1986a,b; Kirby et al., 1995). Blockade of long-term potentiation in hippocampal slices has been demonstrated for several cannabinoid ligands (Norwicky et al., 1987; Collins et al., 1994, 1995) and endogenous cannabinoids (Terranova et al., 1995; Stella et al., 1997). Furthermore, cannabinoid agonists inhibit acetylcholine release in the hippocampus (Gifford and Ashby, 1996; Gessa et al., 1997; Gifford et al., 1997), norepinephrine release from human and guinea pig (but not rat or mouse) hippocampal slices (Schlicker et al., 1997), and presynaptic glutamate release in cultured hippocampal cells (Shen et al., 1996). While cholinergic and noradrenergic neurons project into the hippocampus, circuits within the hippocampus are glutamatergic. Thus, cannabinoids may block transmission both into and within the hippocampus by blocking presynaptic neurotransmitter release. Recently, it was reported that the effects of Δ^9-THC on working memory correlated with increases in dopamine turnover in prefrontal cortex, implicating this as another site for cannabinoid effects on memory (Jentsch et al., 1997).

5.4. Antinociceptive effects

Cannabinoids have antinociceptive effects through both spinal and supraspinal mechanisms (Lichtman and Martin, 1991; Smith and Martin, 1992; Martin et al., 1993; Lichtman et al., 1996). These effects are attributed to cannabinoid receptors in areas that mediate nociceptive neurotransmission, including the dorsal horn of the spinal cord and the periaqueductal gray (Herkenham et al., 1991b). Although similar to opioid analgesia, cannabinoid analgesia is not mediated by opioid receptors since the effects of morphine plus cannabinoids are sometimes synergistic (Welch et al., 1995), and opioid antagonists generally have no effect on cannabinoid-induced antinociception. However, a κ receptor antagonist has been shown to attenuate spinal (Welch, 1993; Smith et al., 1994), but not supraspinal (Welch et al., 1995) cannabinoid antinociception.

Electrophysiological studies have demonstrated that cannabinoids specifically decrease nociceptive neurotransmission both in the lumbar spinal cord (Hohmann et al., 1995) and the ventroposterolateral nucleus of the thalamus of rats (Martin et al., 1996). Behavioral measurements of nociception show significant correlations with the electrophysiological data. Another study indicates that cannabinoids act in the lumbar spinal cord via facilitation of descending noradrenergic pathways from the lateral reticular nucleus (Lichtman and Martin, 1991). Cannabinoid effects on spinal antinociception are mediated by G_i–G_o proteins which decrease cAMP and activate low conductance Ca^{2+}-gated K^+ channels (Welch et al., 1995). Supraspinal mechanisms act at the dorsolateral periaqueductal gray, dorsal raphe nuclei (Martin et al., 1995) and/or posterior ventrolateral periaqueductal gray (Lichtman et al., 1996). Biochemical studies indicate that cannabinoid effects are mediated by inhibitory G proteins which affect 'N'-type Ca^{2+} channels (Welch et al., 1995), but not adenylyl cyclase (Cook et al., 1995; Welch et al., 1995) or K^+ channels (Welch et al., 1995).

6. Chronic effects of Δ^9-THC

6.1. Tolerance

Chronic administration of cannabinoids to animals results in tolerance to many of the acute effects of Δ^9-THC, including memory disruption (Deadwyler et al., 1995c), decreased locomotion (Abood et al., 1993; Oviedo et al., 1993), hypothermia (Pertwee et al., 1993; Fan et al., 1996), neuroendocrine effects (Rodríguez de Fonseca et al., 1991) and analgesia (Adams and Martin, 1996). Although there is little evidence of tolerance in humans resulting from intermittent use, tolerance may develop after prolonged use of high doses (Hollister, 1986).

Several studies have attempted to correlate behavioral tolerance with biochemical alterations, and there is evidence that tolerance to cannabinoids is both pharmacokinetic and pharmacodynamic in nature. Chronic treatment with CP55940 increases the activity of the microsomal cytochrome P450 oxidative system (Costa et al., 1996), suggesting some pharmacokinetic tolerance. Chronic cannabinoid treatments also produce changes in brain cannabinoid receptors and cannabinoid receptor mRNA levels, indicating that pharmacodynamic effects are important as well. Effects on CB1 receptor mRNA are not totally consistent, with chronic cannabinoid treatment producing variable effects on CB1 mRNA levels in the brain (Abood et al., 1993; Rubino et al., 1994; Romero et al., 1997). The finding that effects on CB1 mRNA levels depend on the time course of treatment and varies between brain regions (Zhuang et al., submitted) may explain these disparities.

Brain cannabinoid receptor levels usually decrease after prolonged exposure to agonists (Oviedo et al., 1993; Rodríguez de Fonseca et al., 1994; Fan et al., 1996; Romero et al., 1997), although some studies have reported increases (Romero et al., 1995) or no changes (Abood et al., 1993) in receptor binding in the brain. Appropriate controls have demonstrated

that down-regulation of cannabinoid receptors is homologous, and not simply due to neurotoxicity. Differences among studies may depend on the treatment agonist used, brain region examined or treatment time. For example, levonantradol produces a greater desensitization of adenylyl cyclase inhibition than Δ^9-THC in cultured neuroblastoma cells (Dill and Howlett, 1988), which may be explained by the efficacy differences between these two agonists (Sim et al., 1996a; Burkey et al., 1997). Furthermore, a time course study revealed differences in the rates and magnitudes of receptor down-regulation across brain regions (Breivogel et al., 1998). These findings suggest that tolerance may develop at different rates to different acute effects of cannabinoids.

Chronic treatment with Δ^9-THC also produces variable effects on cannabinoid-mediated signal transduction systems. For example, one study examining the effect of in vivo chronic CP55940 treatments found no change in adenylyl cyclase in cerebellar membranes despite a 50% reduction in receptor binding (Fan et al., 1996), suggesting the presence of receptor reserve. Chronic Δ^9-THC treatment produces significant desensitization of cannabinoid-activated G proteins in a number of rat brain regions, as determined by cannabinoid-stimulated [^{35}S]GTPγS autoradiography (Sim et al., 1996a). Moreover, the time course of the decrease in cannabinoid-stimulated [^{35}S]GTPγS binding varies across brain regions (Breivogel et al., 1998).

It is difficult to extend the findings of these short-term animal studies to human marijuana use. In order to simulate long-term use, the doses used in animal studies are higher than normally achieved by smoking marijuana. For example, the average human may absorb approx. 0.04 mg/kg of Δ^9-THC from a typical marijuana cigarette, compared to 10–20 mg/kg/day used in many chronic studies in rats. In fact, tolerance was only definitive in human experiments when doses were raised to approx. 3 mg/kg/day (Hollister, 1986). Nevertheless, it is likely that some of the same biochemical adaptations to chronic cannabinoid administration occur in both laboratory animals and humans, but the magnitude of the effects in humans may be smaller in proportion to the respective doses used.

6.2. Reward and dependence

Confirmed evidence of cannabinoid physical dependence in animals has been observed only by administration of the CB1 antagonist SR141716A to animals treated chronically with cannabinoids (Aceto et al., 1995; Tsou et al., 1995). This suggests that the long half-life and slow elimination of Δ^9-THC, and its active metabolite 11-OH-Δ^9-THC, may prevent significant abstinence symptoms under normal cannabis use. The precipitated withdrawal effects produced by SR141716A have some of the characteristics of opiate withdrawal, but are not affected by opioid antagonists and display different motor system effects.

Cannabinoids are not generally self-administered by animals (Dewey, 1986) indicating that they are not reinforcing. However, recent studies have indicated that cannabinoids increase dopamine levels in the mesolimbic dopamine system of rats (Chen et al., 1993; Tanda et al., 1997), a pathway associated with reinforcement (Di Chiara and Imperato, 1988). These increases in dopamine are due to increases in the firing rate of dopamine cells in the ventral tegmental area by Δ^9-THC, which were both dose-dependent and SR141716A-reversible (French, 1997). However, these increases in firing rate in the ventral tegmental area could not be explained by increases in the firing of the A10 dopamine cell group, where other abused drugs have been shown to act (Gifford et al., 1997).

A recent study in rats indicated that the behavioral cannabinoid withdrawal syndrome correlates with stimulation of central amygdaloid corticotropin releasing hormone release, consistent with the consequences of withdrawal from other abused drugs (Rodríguez de Fonseca et al., 1997). However, the relevance of this data to human cannabinoid abuse is unknown, since the withdrawal syndrome for cannabinoids (Aceto et al., 1995; Tsou et al., 1995) and corresponding increase in corticotropin releasing hormone (Rodríguez de Fonseca et al., 1997) are only observed following administration of the antagonist SR141716A to cannabinoid-tolerant animals. Furthermore, acute administration of Δ^9-THC also produces increases in corticotropin releasing hormone and adrenocorticotropin release (Jackson and Murphy, 1997). This may explain the aversive effects of cannabinoids in animals, and may indicate that the increase in corticotropin releasing hormone is merely a rebound effect. Thus, while cannabinoids appear to be conforming to some of the neurobiological effects of other drugs abused by humans, the underlying mechanisms of these actions and their significance in determining the reinforcement and dependence liability of cannabinoids are still undetermined.

7. Future directions

Despite the tremendous progress in understanding the pharmacology and neurobiology of brain cannabinoid systems, this field is still in its developmental stages. In the area of drug development, future progress should provide more specific agonists and antagonists for CB1 and CB2 receptors, with varying potential for therapeutic uses. A key focus for future

study is the neurobiology of endogenous cannabinoids: establishing the precise brain localization, storage/release mechanisms, and uptake mechanisms will be crucial in determining the biological role of this system. Technology that will be crucial in establishing the biological significance of these systems will be the construction of transgenic knockout mice, as already accomplished for various opioid receptor types (Childers, 1997). In 1997, both CB1 and CB2 receptor knockout mice were generated, but no published reports have yet appeared. Finally, it will be important for future studies to firmly establish the abuse potential for cannabis compared to other drugs of abuse. Recent studies which demonstrate both cannabinoid withdrawal syndromes as well as reinforcement mechanisms provide interesting clues to the acute and chronic actions of this drug, but the relevance of these findings to normal usage patterns in humans has not yet been established. The design of well-controlled clinical studies with not only cannabis itself but also potent synthetic analogs may help to answer these important questions.

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