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THE EFFECTS OF CANNABINOIDS ON THE BRAIN

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Abstract—Cannabinoids have a long history of consumption for recreational and medical reasons. The primary active constituent of the hemp plant *Cannabis sativa* is Δ^9-tetrahydrocannabinol (Δ^9-THC).

In humans, psychoactive cannabinoids produce euphoria, enhancement of sensory perception, tachycardia, antinociception, difficulties in concentration and impairment of memory. The cognitive deficiencies seem to persist after withdrawal. The toxicity of marijuana has been underestimated for a long time, since recent findings revealed Δ^9-THC-induced cell death with shrinkage of neurons and DNA fragmentation in the hippocampus.

The acute effects of cannabinoids as well as the development of tolerance are mediated by G protein-coupled cannabinoid receptors. The CB1 receptor and its splice variant CB1A, are found predominantly in the brain with highest densitities in the hippocampus, cerebellum and striatum.

The CB2 receptor is found predominantly in the spleen and in haemopoietic cells and has only 44% overall nucleotide sequence identity with the CB1 receptor. The existence of this receptor provided the molecular basis for the immunosuppressive actions of marijuana. The CB1 receptor mediates inhibition of adenylate cyclase, inhibition of N- and P/Q-type calcium channels, stimulation of potassium channels, and activation of mitogen-activated protein kinase. The CB2 receptor mediates inhibition of adenylate cyclase and activation of mitogen-activated protein kinase.

The discovery of endogenous cannabinoid receptor ligands, anandamide (*N*-arachidonylethanolamine) and 2-arachidonylglycerol made the notion of a central cannabinoid neuromodulatory system plausible. Anandamide is released from neurons upon depolarization through a mechanism that requires calcium-dependent cleavage from a phospholipid precursor in neuronal membranes. The release of anandamide is followed by rapid uptake into the plasma and hydrolysis by fatty-acid amidohydrolase.

The psychoactive cannabinoids increase the activity of dopaminergic neurons in the ventral tegmental area-mesolimbic pathway. Since these dopaminergic circuits are known to play a pivotal role in mediating the reinforcing (rewarding) effects of the most drugs of abuse, the enhanced dopaminergic drive elicited by the cannabinoids is thought to underlie the reinforcing and abuse properties of marijuana.

Thus, cannabinoids share a final common neuronal action with other major drugs of abuse such as morphine, ethanol and nicotine in producing facilitation of the mesolimibic dopamine system.

CONTENTS

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ABBREVIATIONS

AM404 *N*-(4-hydroxyphenyl)arachidonylamide
cAMP Adenosine 3′:5′-cyclic monophoshate
CRF Corticotropin releasing factor
EC_{50} Half-maximally effective concentration
GABA γ-Aminobutyric acid
IBMX 3-Isobutyl-1-methyl-xanthine
IC_{50} Half-maximally inhibitory concentration
LTP Long-term potentiation
MAP kinase
Mitogen-activated protein kinase
NMDA *N*-Methyl-D-aspartate
[^{35}S]GTPγS 5′-[γ-[^{35}S]thio]-triphosphate
SR1417116
N-Piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorphenyl)-4-methyl-3-pyrazole-carboxamide
SR144528 *N*-[(1*S*)-endo-1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-1(4-methylbenzyl)-pyrazole-3-carboxamide
Δ^9-THC Δ^9-Tetrahydrocannabinol
TNFα Tumor necrosis factor α
VTA Ventral tegmental area
WIN55 212-2
R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolol[1,2,3-de]-1,4-benzoxazinyl]-(1-naphtalenyl)methadone

1. INTRODUCTION

The hemp plant, *Cannabis sativa*, has been used for over 4000 years as a recreational drug due to its mind-altering effects. Marijuana, the common name for cannabis, is by far the most commonly used street drug world wide today. However, since the 4th century BC, it also has been employed as a therapeutic drug. No other drug of abuse arouses greater controversy than cannabis. The public debate centers upon the possible legalization of the cannabis use, at least for therapeutic use. There are a number of well-proofed and potential therapeutic actions of cannabinoids, such as antiemetics, analgesia, anticonvulsant action and lowered intraoccular pressure (Hollister, 1986). More recently, its use as an appetite stimulant has been indicated in patients with cachexia or wasting disease such as AIDS (Timpone *et al*., 1997; Volicer *et al*., 1997). Unquestionably, existence of psychoactive effects, development of tolerance and the abuse potential of cannabinoids have discouraged their therapeutic use.

Due to the cloning and characterization of specific cannabinoid receptors, the discovery of endogenous cannabimimetic compounds and the availability of selective receptor antagonists, our knowledge of the mechanisms of action of cannabinoids has been greatly increased during the last few years. It is the aim of the present review to present the most important breakthroughs which leads to a better understanding of the actions and risks of cannabinoids. The principle psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), as well as structurally related cannabinoids are highly lipophilic molecules which exert their central effects by binding at specific membrane receptors, the CB1 cannabinoid receptors. Beside their central effects, cannabinoids possess several peripheral effects, which are believed to be mediated by another type of cannabinoid receptor, the CB2 receptor. Recent advances in characterizing these cannabinoid receptors as well as their signal transduction mechanisms involved will be presented in the present review. A further concern will be the import discovery of the endogenous cannabimimetic system in the brain. The possible physiological role of the two endogenous cannabimimetic compounds, the eicosanoid anandamide (Devane *et al*., 1992), and the more recently identified 2-arachidonylglycerol (Mechoulam *et al*., 1995; Stella *et al*., 1997) will be discussed with regard to a potentially modulation of pain perception and memory.

2. PHARMACOLOGY OF CANNABIS

2.1. Active Ingredients

The flowering tops and leaves of the plant *C. sativa* secret a resin containing about 60 terpenophenolic compounds which are called cannabinoids. The highest amount of cannabinoids has been found in the flowering tops, followed by the leaves, whereas only small amounts are found in stem and roots. The seed does not contain any cannabinoids. Preparations, such as marijuana, which is made from dried leaves and tops of the plant, have a lower cannabinoid content than hashish, which is a preparation from the dried resin itself.

The mechanism of action of marijuana remained unclear until chemical analyses of plant extracts

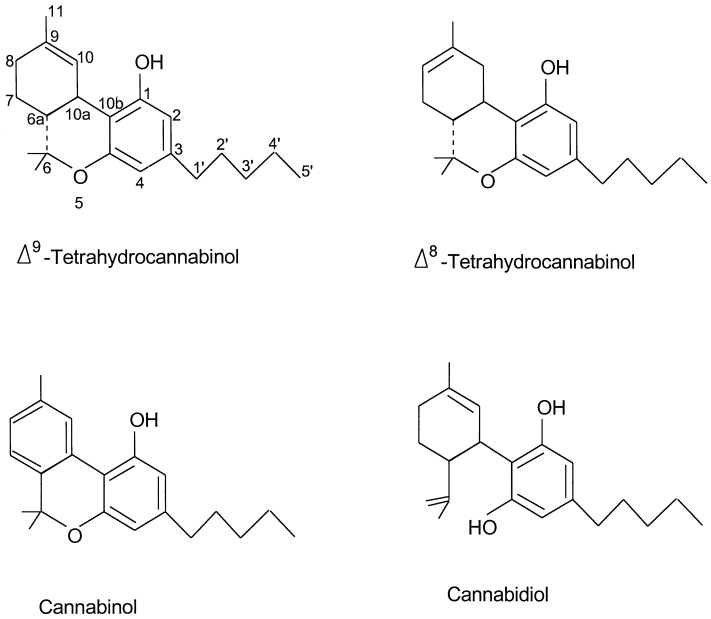

Fig. 1. Structures of four compounds of the hemp plant, *Cannabis sativa*: the psychoactive Δ^9-THC, Δ^8-THC and cannabinol as well as the non-psychoactive cannabidiol.

revealed a principle active ingredient, Δ^9-THC, and identified its chemical structure (Gaoni and Mechoulam, 1964). The potency of street marijuana has increased progressively during the past years. In 1974, the average Δ^9-THC content of confiscated marijuana was 0.35%, but had risen to 3.54% in 1990 (Perez-Reyes *et al.*, 1991). The marked lipophilic properties of Δ^9-THC allows the passage across the blood–brain barrier. In addition to Δ^9-THC, several less potent metabolites and related compounds, such as the also psychoactive Δ^8-THC and cannabinol as well as the non-psychoactive cannabidiol (Fig. 1) are also found in the *Cannabis* plant, but much less is known about their pharmacological properties. Several hundred additional compounds are produced by pyrolysis when marijuana is smoked, which may contribute to the psychoactive effects of marijuana [for review see Tjeerdema (1987)]. While most of the other cannabinoids found in marijuana are either inactive or only weakly active, they have the potential of interacting with Δ^9-THC to either increase or decrease its potency.

Since Δ^9-THC is highly lipophilic, it has been suggested that some of the effects of cannabinoids are due to non-specific disruption of cellular membranes, which may stimulate or inhibit membrane-associated enzymes and alter ion channels (Hillard *et al.*, 1985; Martin, 1986). However, the enantiomeric selectivity and structural requirements for physiological activity suggest that the cannabinoids exert most of their actions by binding at a specific membrane receptor (see Section 3). The (−)-*trans* isomer is more potent then the (+)-*trans* isomer in tests of monkey behavior, dog static ataxia, mouse hyperthermia, analgesia, and spontaneous activity (Edery *et al.*, 1971; Jones *et al.*, 1974; Uliss *et al.*, 1975; Martin *et al.*, 1981). Extensive structure–activity relationship studies have been undertaken, in order to synthesize and evaluate cannabinoid analogs with the aim of separating desired pharmacological effects from unwanted psychoactive effects. These investigations led to the development of a model for ligand interaction with a specific cannabinoid receptor using antinociception as biological activity (Johnson and Melvin, 1986; Melvin *et al.*, 1993). The results of these studies imply that the Δ^9-THC molecule attaches to the receptor with three points:

1. the lipophilic alkyl side chain at C3;
2. a free phenolic hydroxyl group; and
3. an appropriate substituent at the carbocyclic ring C9.

In vivo, Δ^9-THC is rapidly absorbed and converted in lungs and liver into a centrally active metabolite, 11-hydroxy-Δ^9-THC (Abood and Martin, 1992). This metabolite is more potent than Δ^9-THC and more readily cross the blood–brain barrier. Finally, 11-hydroxy-Δ^9-THC is converted in the liver to several inactive metabolites, including 11-nor-carboxy-Δ^9-THC, which is the most abundant metabolite in plasma and urine and can be detected 2–3 days following smoking a single marijuana cigarette.

2.2. Effects of Cannabinoids

Although cannabinoids have been shown to exert direct cellular effects on a number of organs, including the immune system, the liver and reproductive system, the most of the behavioral and pharmacological effects studied till now appear to involve the central nervous system.

2.2.1. *Euphoria, Cognition and Behavior*

Behavioral effects may vary as a function of dose, route of administration, expectations of subjects, and individual vulnerability to certain effect. Despite the influence of these factors, the cannabinoids have

been shown to produce a unique syndrome of effects on the behavior of humans and animals. The most prominent feature is an initial period of euphoria and relaxation, which is followed by a depressant period. At low doses, cannabinoids produce a mixture of stimulatory and depressant effects, and at higher doses they exert predominantly depression. The pleasurable subjective effects of marijuana smoking, which presumably contribute to its abuse, include furthermore the feeling of tranquillity and distortions in both hearing and vision. Lukas *et al.* (1995) demonstrated electroencephalographic correlates of marijuana-induced euphoria. Subjects smoking marijuana cigarettes with 1.26 or 2.53% Δ^9-THC reported multiple episodes of intense good effects or euphoria during the first 15 min. These episodes of euphoria were accompanied by a rapid increase in plasma Δ^9-THC. The EEG alpha power was significantly higher during the episodes of euphoria, while the beta activity was decreased. These findings suggest that the transient EEG changes may reflect a neurophysiological correlate of the reinforcing effects of marijuana. In chronic marijuana users with an exposure of at least 15 years, Struve *et al.* (1994) have found characteristic persistent increases in frontal-central theta activity as well as persistent increases in alpha power over frontal areas.

Acute consumption of marijuana is associated with impaired functioning in a variety of cognitive and performance tasks, including impaired memory, altered time sense and decrements in tasks such as reaction time, learning perception, motor coordination and attention (Block *et al.*, 1992; Chait and Perry, 1994; Heishman *et al.*, 1997; Court, 1998). Subjective, physiological and performance effects of marijuana can persist for up to 2 days (Heishman *et al.*, 1990; Yesavage *et al.*, 1985). Cannabis is one of the drugs most likely to be involved in motor car accidents and traffic fatalities (Robbe, 1994; Buchan *et al.*, 1998). At doses equivalent to one or two marijuana cigarettes, processes involved in car driving are impaired for up to 24 hr; this means, long after the user perceives the subjective effects of the drug. Areas impaired include coordination, tracking, perception, vigilance and performance in both driving simulators and on the road (Moskowitz, 1985). Cannabis is often coabused with other drugs, such as alcohol. The cognitive impairment produced by alcohol and marijuana has been reported to be additive (Perez-Reyes *et al.*, 1988). The effects of both drugs on the impairment of cognitive abilities are remarkably comparable (Chait and Perry, 1994; Heishman *et al.*, 1997). In general, one of the most consistently reported behavioral effects of marijuana in humans and animals is the disruption in the free recall of previously learned items and other memory processes. Recent research using radial arm and delayed match to sample tasks has implicated cannabinoid receptors in the hippocampus which mediate the deficits in short-term memory by cannabinoids (Heyser *et al.*, 1993; Lichtman and Martin, 1996). Therefore, it seems to be likely that Δ^9-THC impairs acquisition and working memory but not retrieval processes.

In various animal models, cannabinoids have been shown to produce a tetrad of behavioral effects, that is, a decrease in spontaneous locomotor activity as well as hypothermia, antinociception and catalepsy (Pertwee and Ross, 1991). These pharmacological effects have been proven to be excellent predictors of cannabimimetic activity in rodents (Compton *et al.*, 1991). Several brain regions may be associated with the modulation of locomotor activity by the cannabinoids, such as the basal ganglia (Gough and Olley, 1978; Moss *et al.*, 1981) and the cerebellum (Martin *et al.*, 1981, 1991). The behavioral effects of the cannabinoids within the basal ganglia seems to occur in concert with several other neuromodulators such as dopamine, acetylcholine and γ-aminobutyric acid (GABA) (Pertwee and Greentree, 1988; Pertwee *et al.*, 1988; Pertwee, 1991; Souilhac *et al.*, 1995; Maneuf *et al.*, 1996; Romero *et al.*, 1998a,b. The effects of cannabinoids within the basal ganglia will be discussed in more detail in Section 5.1. Clear differences in behavior, however, are observed at low and high concentrations of Δ^9-THC and other cannabinoids. Low doses of Δ^9-THC are stimulatory followed by sedation, whereas high doses induce only sedation, suggesting different mechanisms of action at high and low concentration of agonist.

2.2.2. *Antinociceptive Effects of Cannabinoids*

One of the most well-characterized biological effects of cannabinoids, such as Δ^9-THC, is their capability of inhibiting nociception, that is, pain transmission. It has been reported previously that the tetrahydrocannabinols derived from marijuana were found to have only moderate analgesic activity when tested in mice by the hot-plate test (Wilson and May, 1975). In contrast, of the several metabolites of these cannabinoids tested by the authors, the 11-hydroxy derivatives were more potent than the parent compounds. This suggests that metabolism to 11-hydroxy congeners may be necessary for the mediation of analgesic activity in the mouse hot-plate test but probably not for other pharmacologic effects produced by these substances. The antinociceptive properties of cannabinoids manifested themselves as a potent, long-lasting elevation of tail-flick latencies in rodents. The mechanisms of action for cannabinoid antinociception include both spinal and supraspinal actions. In an elegant study, cannabinoids have been shown to suppress noxious stimulus-evoked activity of identified nociresponsive neurons of the ventroposterolateral nucleus of the thalamus in a dose-dependent and reversible manner (Martin *et al.*, 1996). It is intriguing that cell firing evoked by noxious stimuli was affected more than spontaneous firing. Taken together, these findings suggest that cannabinoids decrease nociceptive neurotransmission at the level of the thalamus. More recently, it has been shown that the tail flick latency of rats, which received a direct injection of cannabinoids into the rostral ventromedial medulla, was increased by $>50\%$ (Martin *et al.*, 1998). This raises the possibility that the actions of cannabinoids as well as of the endogenous cannabinoid system (see below) in the rostral ventromedial medulla

may modulate nociceptive responsiveness. Lichtman *et al.* (1996) have reported that microinjection of cannabinoids in the periaqueductal gray, in the region of the dorsal raphé, produced antinociception, which is completely prevented by coapplication of pertussis toxin, consistent with an involvement of a G protein (see Section 3.1.2). In contrast, dibutyryl adenosine 3′:5′-cyclic monophoshate (cAMP) failed to attenuate cannabinoid-induced antinociception. The periaqueductal gray is involved in ascending pain transmission, since it receives afferents from nociceptive neurons in the spinal cord and sends projections to thalamic nuclei that process nociception. The periaqueductal gray is also a major component of a descending pain inhibitory system. Activation of this system inhibits nociceptive neurons in the dorsal horn of the sinal cord (Behbehani, 1995).

As shown by Lichtman and Martin (1991a), the antinociception induced by systemically administered cannabimimetic compounds was significantly attenuated by spinal transection. This indicates that the mechanisms of action for the cannabinoid-induced analgesia include both spinal and supraspinal actions. Furthermore, it has been shown in tail flick studies that the cannabinoid receptor system participates in the descending noradrenergic control of nociception. Inhibition of the descending system inhibits the mean discharge rates of the nociceptive neurons in the dorsal horn of the spinal cord. These descending effects are mediated by the neurotransmitters noradrenaline and serotonin. The antinociceptive action of cannabinoids seems to be closely related to the noradrenergic system. Rats received an intravenous (i.v.) injection of Δ^9-THC and subsequently were given an intrathecal (i.t.) injection of either the selective serotonin receptor antagonist, methysergide or the α_2-noradrenergic receptor agonist, yohimbine. Whereas yohimbine significantly reversed the cannabinoid-induced elevation of tail-flick latencies, methysergide had no effect (Lichtman and Martin, 1991b). This finding implies that Δ^9-THC activates descending inhibitory α_2-noradrenergic neurons which project from the brain stem to the spinal cord and which are known to inhibit nociception transmission at the spinal level [for review see Willis and Westlund (1997)]. Moreover, Lichtman and Martin (1991b) have found that the antinociception induced by Δ^9-THC was significantly reduced when yohimbine was administered in the lumbar region; however, administration in the upper thoracic region failed to have an effect.

To reiterate, the potent analgesic effects of cannabimimetic drugs involve pain-processing areas of the brain and spinal cord. Moreover, due to their sensitivity to the CB1 receptor antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorphenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A) there is strong evidence that the cannabinoid-induced antinociception is mediated by the CB1-type cannabinoid receptor (see Section 3.1). However, recently a cannabinoid-induced attenuation of pain has been produced by chemical damage to cutaneous tissue has been reported (Calignano *et al.*, 1998). It is intriguing that the attenuation of pain produced by tissue damage was sensitive to both CB1 and CB2 receptor antagonist, indicating an interaction with CB1 and CB2 receptors located outside the CNS.

The well-known antinociceptive effects of opioids are mediated by μ opioid receptors. Several experiments have been performed, in order to investigate a possible interaction between the opioid system and the cannabinoids. It has been shown previously that i.t. administration of Δ^9-THC subsequently to pretreatment with morphine results in a greater than additive antinociceptive effect (Welch and Stevens, 1992; Welch *et al.*, 1995; Smith *et al.*, 1998a). The increase in the potency of morphine was independent of the routes of application of Δ^9-THC and morphine. Furthermore, the ability of the CB1 receptor antagonist SR141716A to antagonize the enhancement of morphine by Δ^9-THC indicates that Δ^9-THC was acting through a cannabinoid receptor mechanism. Surprisingly, pretreatment of mice with the selective μ opioid receptor antagonist, naloxone, failed to block the antinociceptive effects of various cannabinoids, indicating that the cannabinoid-induced antinociception does not occur due to direct interaction with the μ opioid receptor (Welch and Stevens, 1992). Several findings have implicated dynorphins in the antinociceptive actions of Δ^9-THC and its enhancement of morphine-induced antinociception. In contrast to naloxone, the antinociceptive effect of cannabinoids could be attenuated by i.t. injection of norbinaltorphimine, a κ opioid antagonist, but not by μ and δ opioid receptor antagonists naloxone and ICI 174 864, respectively (Welch, 1993).

Furthermore, subthreshold doses of the κ opioid receptor agonist, dynorphin A (1–8), enhanced the antinociception induced by Δ^9-THC. Inhibition, however, of the enzymatic conversion of dynorphin A (1–8) into the δ opioid receptor agonist, [leu5]-enkephalin, has been shown to attenuate the enhancement of morphine-induced antinociception by Δ^9-THC (Pugh *et al.*, 1996). The overadditive effect of morphine and Δ^9-THC was also attenuated by antisera to dynorphin A (1–8). Moreover, these authors demonstrated that the Δ^9-THC-induced antinociceptive effect was blocked by both κ and δ receptor antagonists. These findings suggests that the antinociception of morphine, which is predominantly mediated by μ receptors, is enhanced by Δ^9-THC through the activation of κ and δ opioid receptors.

Smith *et al.* (1994b) performed chronic administration studies in mice, in order to determine if cross tolerance could be established between Δ^9-THC and the κ opioid receptor. The chronically Δ^9-THC-treated mice were significantly tolerant, not only tolerant to the Δ^9-THC-induced antinociception in the tail-flick test, but also to the κ opioid agonist compared with those treated chronically with vehicle suggesting the existence of cross-tolerance for Δ^9-THC and κ opioid agonists. This finding implies that Δ^9-THC and the κ opioid agonists may share a common mechanism of action in the production of antinociception, but that Δ^9-THC does not act directly at the κ receptor.

Taken together, these findings may indicate that a neuronal pathway involving κ opioid receptors may be distal to or converging with a pathway involving

cannabinoid receptors in spinal analgesia, whereas the μ receptor seems not to be directly involved. It is intriguing that κ opioid receptor antagonists, which attenuate the antinociceptive effect of Δ^9-THC, did not affect other commonly observed cannabinoid actions, which included hypothermia, hypoactivity and catalepsy (Welch, 1993). It should be emphasized, that this finding demonstrates that cannabinoid actions can be distinguished from each other. The pharmacological separation of antinociception from the other cannabinoid-induced actions implies that it may have a mechanism distinct from other effects.

2.2.3. *Anticonvulsive Effects of Cannabinoids*

Available evidence suggest that various cannabinoids possess some antiepileptic properties in humans and in animal seizure models (Cunha *et al*., 1980; Carlini and Cunha, 1981; Karler and Turkanis, 1981). 'Epilepsies' are no single neurological disorder, but rather a group of disorders. From a pharmacological point of view, three distinct forms of the disorder may be distinguished:

1. petit mal (absence) seizures, which are a generalized type of epilepsy and well suppressed by a group of anticonvulsants whose prototype is ethosuximide;
2. generalized tonic-clonic (grand mal) and cortical focal seizures, which respond to the drug group represented by phenytoin and carbamazepine; and
3. temporal lobe seizures (partial seizures with complex symptomatology), which tend to be refractory to any therapy, except for a limited efficacy associated only with the phenytoin group of drugs.

The complex partial seizures are the most frequent type of seizures in humans and occur in ca 40–50% of all patients with epilepsy [for review see Löscher (1997)]. Albeit the management of other types of epilepsy is possible by means of a single anticonvulsant, difficulties arise in the treatment of the complex partial types.

Although cannabinoids have been shown to possess anticonvulsant properties, the problem of determining the therapeutic potential of the cannabinoids as antiepileptics is basically the same as that of determining their potential for any other therapeutic purpose, for instance analgesia. The main problem concerns the separation of psychoactive from anticonvulsant activity. However, of those cannabinoids devoid of psychotropic actions, cannabidiol has been reported to possess anticonvulsants effect comparable with those of phenytoin (based on similar spectra of anticonvulsant activity) in mice with electroshock convulsions (Karler and Turkanis, 1981). Furthermore, these authors reported that cannabidiol blocks limbic seizures in mice. This is intriguing, since the limibic system is considered to be closely associated with complex partial seizures. Moreover, clinical trials with cannabidiol were undertaken in patients with complex partial seizures with secondary generalization (Cunha *et al*., 1980; Carlini and Cunha, 1981). The data reveal that cannabidiol in oral doses of 200–300 mg day^{-1} is, in fact, effective against this form of epilepsy.

It is intriguing that cannabinoids have been shown to interact with the glutamatergic transmission in the CNS. Glutamate is a neurotransmitter that mediates fast neuronal excitation in a majority of synapses in the central nervous system. Glutamate stimulates both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. While activation of NMDA receptors has been implicated in a variety of neurophysiologic processes, excessive NMDA receptor stimulation (excitotoxicity) is thought to be primarily responsible for neuronal injury in a wide variety of acute neurological disorders including hypoxia-ischemia, seizures, and trauma. The synthetic and non-psychotropic cannabinoid, dexanabinol (HU-211), blocks NMDA receptors in a stereospecific manner and that the interaction occurs at binding sites distinct from those of other non-competitive NMDA antagonists or of glutamate and glycine (Feigenbaum *et al*., 1989). Moreover, HU-211 induced stereotype and locomotor hyperactivity in mice and tachycardia in rat, effects typically caused by NMDA receptor antagonists. HU-211 is also a potent blocker of NMDA-induced tremor, seizures, and lethality in mice. Furthermore, this compound was reported to be useful as a non-psychoactive drug that protects against NMDA-receptor-mediated neurotoxicity (Belayev *et al*., 1995).

More recently, Shen *et al*. (1996) provided electrophysiological evidence that cannabinoids are capable of inhibiting presynaptic release of glutamate in rat hippocampal cultures, whereas they failed to alter the GABAergic synaptic transmission. Glutamate is the major excitatory neurotransmitter in the brain. Most neurons receive glutamatergic input, and many neurotransmitters produce presynaptic inhibition of glutamatergic synaptic transmission. For these reasons, it is possible that presynaptic inhibition of glutamate release by the cannabinoid receptor/ligand neuromodulatory system accounts for their potential clinical application as anticonvulsants and, as reported above, as analgesics.

2.2.4. *Other Biological Effects*

As already mentioned above, the most prominent effects of cannabinoids, that is, the psychoactive, antinociceptive and antiepileptic effects are mediated by their action on the central nervous system. Beside these central actions, however, cannabinoids and cannabimimetic agents are known to exert a variety of peripheral actions, too. The most important, which should be briefly described, are cardiovascular and immunosuppressive effects.

Early studies with volunteer marijuana smokers reported that Δ^9-THC can induce tachycardia, orthostatic hypotension and decreased platelet aggregation (Clark *et al*., 1974; Schaefer *et al*., 1979; Merritt *et al*., 1980). The cardiovascular effects of marijuana smoking become obvious by the typical conjunctivae redden which is due to the dilatation of blood vessels and increased heart rate with a concomitant peripheral vasodilatation (Dewey, 1986). In the electrocardiogramm, the P and T waves are

changed and the ST segment is decreased (Johnson and Domino, 1971). Therefore, in persons with pre-existing cardiovascular diseases, marijuana smoking is to be expected to aggravate the symptoms of their diseases. These include individuals with congestive heart failure and angina. In rats, Δ^9-THC can induce a transient pressor response which is followed by hypotension and bradycardia (Dewey, 1986). More recent experiments (Varga *et al*., 1996; Lake *et al*., 1996) have indicated that the CB1 cannabinoid receptor is involved in the hypotension induced by both Δ^9-THC and the endogenous cannabimimetic compound anandamide (see Section 4.1). The hypotensive response was inhibited by the selective CB1 receptor antagonist, SR141716A, and attenuated by either cervical spinal cord transection or by α-adrenergic receptor antagonists (Varga *et al*., 1996). The authors have concluded that the depressor response is due to an inhibition of the sympathetic tone mediated by CB1 receptors. Ishac *et al*. (1996) investigated the effects of Δ^9-THC, anandamide and the selective CB1 receptor antagonist, SR141716A, on the exocytotic release of noradrenaline in rat isolated atria and vasa deferentia, in order to determine if the hypotension is related to presynaptic inhibition of noradrenaline release. Both Δ^9-THC and anandamide were shown to abolish [^{3}H]noradrenaline release in this study in a concentration-dependent manner (0.3–10 μM). Furthermore, the inhibitory effects of both compounds were antagonized by SR141716A. These findings suggest that activation of presynaptic CB1 receptors located on peripheral sympathetic nerve terminals mediate the sympathoinihibitory effects of cannabinoids. More recently, it has been reported that the CB1 receptor antagonist, SR141716A, elicit an increase in blood pressure in rats subjected to haemorrhagic shock, whereas it failed to do so in normotensive rats (Wagner *et al*., 1997). Furthermore, intracerebroventricular (i.c.v.) administration of the antagonist during shock did not affect the blood pressure. Blood from haemorrhaged rats caused hypotension in normal rats, which was shown to be prevented by SR141716A. This finding indicates that the haemorrhagic hypotension is mediated by an activation of peripheral CB1 cannabinoid receptors. Moreover, Wagner *et al*. (1997) have shown that macrophages and platelets from haemorrhaged rats elicit CB1 receptor-mediated hypotensin in normotensive recipients which is accompanied by an increased synthesis of the endogenous cannabimimetic compound, anandamide, by the macrophages. Taken together, there is strong experimental evidence that cannabinoids as well as endogenous cannabinoid receptor ligands cause a hypotension which is mediated by peripheral CB1 receptors.

In addition, a second peripheral action of cannabinoids is the impairment of the immune system [for review see Klein *et al*. (1998)]. Cannabinoids are thought to exert their diverse effects on immunocompetence through both cannabinoid receptors and non-receptors mechanisms. In general, high doses or concentrations (in the millimolar range) of Δ^9-THC exert immunosuppressive effects on both lymphocyte function, including stimulation of proliferation and enhanced production of interleukin-2, and macrophage function (Kaminski *et al*., 1992; Friedman *et al*., 1994). Moderate doses of Δ^9-THC have been reported to suppress the formation of antibodies, to reduce spleen weight and to inhibit the production of interferon (Friedman *et al*., 1994). Cannabinoids have been shown to alter the function and morphology of macrophages and T helper cells from monkeys exposed to cannabis for 1 year (Cabral *et al*., 1991). The number of vacuoles was increased, whereas the phagocytotic and spreading ability was reduced (Lopez-Cepero *et al*., 1986; Cabral *et al*., 1991; Spector and Lancz, 1991; Klein *et al*., 1998).

Unfortunately, definitive data which directly link marijuana use to increased susceptibility to infection in humans currently is unavailable. More studies are needed to determine the role of the cannabinoid receptor/ligand system in immune regulation and homeostasis. However, cumulative reports indicating an immunosuppressive action *in vitro* and in animals support the hypothesis that cannabinoids may modulate the immune system in humans and enhance the disease process.

2.3. Tolerance, Dependence and Toxicity

Tolerance develops to most pharmacological effects of Δ^9-THC but also to other cannabinoids, such as 11-hydroxy-Δ^9-THC. Tolerance has been shown to occur to antinociception, anticonvulsant activity, catalepsy, depression of locomotor activity, and hypotension on repeated administration of Δ^9-THC and other psychoactive cannabinoids (Pertwee, 1991; Abood and Martin, 1992). The development of tolerance follows different time courses and has different extents. Full tolerance appears for hypomobility, whereas analgesia was only reduced by 60% (Rubino *et al*., 1997).

The exact mechanism leading to tolerance is still unknown. In general, tolerance to drugs can occur by two major ways:

1. changes in pharmacokinetics (i.e. absorption, distribution, metabolism and excretion);or
2. changes in pharmakodynamics.

While the first plays only a minor role in the development of drug tolerance, pharmacodynamic events, such as receptor down regulation, receptor conformational change and receptor internalization, are well known to attribute to the development of tolerance. The consequence of changes on receptor level is a decrease in the interaction of ligand and receptor.

Cannabinoid tolerance appears to develop in absence of pharmacokinetic changes. Neither metabolic disposition of the drug, nor the bioavailability of Δ^9-THC derived from cannabis was altered in daily marijuana smoking humans (Perez-Reyes *et al*., 1991). However, Costa *et al*. (1996) reported that the synthetic cannabinoid, CP55 940, induces behavioral tolerance after prolonged treatment in rats, and that this tolerance involves the hepatic drug-metabolizing enzyme system. This implies that the development of the behavioral tolerance in rats

may be at least in part a consequence of modified biotransformation activities.

There is strong evidence that the cannabinoid receptor plays the major part in the production of cannabinoid tolerance, this means, that tolerance is predominantly the consequence of pharmacodynamic events. A frequent phenomena following exposure to drugs for a long period of time is receptor internalization. Internalization of receptor proteins means that receptors on the cell membrane are removed into the cytoplasma, where they are either degraded or recycled. The consequence of the internalization is a decrease in the number of receptors at the cell surface and, in turn, a decrease in binding of ligands to the remaining receptors. On the other hand, the cell can reduce the number of receptors which it makes and, thereby, downregulate the receptors. Indeed, development of tolerance to Δ^9-THC which is accompanied by downregulation of cannabinoid receptors has been observed in the striatum, cerebellum and limbic forebrain, but not in the ventral mesencephalon (Oviedo *et al*., 1993; Rodriguez de Fonseca *et al*., 1994). But although CB1 receptor binding decreases after chronic Δ^9-THC exposure in the most brain regions, this seems not to be accompanied by parallel decreases in CB1 receptor mRNA levels (Romero *et al*., 1997). This might indicate that the primary action of Δ^9-THC would be on the receptor protein itself rather than on the expression of CB1 receptor gene. Recent results indicate that besides the receptor downregulation, a reduction of $G\alpha_i$ expression occurs in the forebrain (63%), cerebellum (58%) and mesencephalon (38%) of tolerant rats (Rubino *et al*., 1997, 1998). Thus, alteration of G protein expression could represent a molecular event associated with the development of cannabinoid tolerance.

In light of the fact that most drugs which are used for recreational purposes produce some form of physiological dependence and that development of tolerance frequently occurs in conjunction with dependence, it seems likely that physical dependence would develop following chronic exposure to marijuana. One of the most usual experimental methods for demonstrating dependence is the abrupt termination of chronic administration of the drug and the observation of withdrawal symptoms. Although tolerance to cannabinoids has been well established and several strategies for assessing dependence liability of drugs in laboratory animals have been developed, studies conducted with Δ^9-THC applied for a long time could provide no consistent evidence that dependence occurs with cannabinoids. A clear-cut abstinence syndrome has been rarely reported, presumably because of the long-life of cannabinoids (see Section 2.2.1), which precludes the emergence of abrupt abstinence symptoms (although nervousness, tension, restlessness, sleep disturbances and anxiety have been described in humans, monkeys, and rats after termination of long-term cannabinoid administration). However, since the discovery of the selective cannabinoid CB1 receptor antagonist, SR141716A, by Rinaldi-Carmona *et al*. (1994), intriguing findings have been presented concerning a possible development of cannabinoid dependence and withdrawal. Recent studies demonstrated that in mice made tolerant to Δ^9-THC, the administration of SR141716A after the last Δ^9-THC injection promptly precipitated a profound withdrawal syndrome (Cook *et al*., 1998). Typical withdrawal behavior became obvious as an increase in paw tremors and head shakes that was accompanied with a decrease in normal behavior such as grooming and scratching. These findings are consistent with SR141716A-precipitated withdrawal in rats (Rubino *et al*., 1998). Beside the behavioral aspects, Rubino *et al*. investigated also biochemical aspects of the cannabinoid withdrawal syndrome in cannabinoid tolerant rats. They observed changes of CB1 receptors and of the α subunit of G proteins throughout the brain during the withdrawal syndrome by use of *in situ* hybridization. *In situ* hybridization after the first SR141716A injection showed that CB1 receptor and G protein α subunits, whose levels were low in tolerance, recovered to their basal level of expression. Thus, the general desensitization of the cannabinoid receptor and of the transduction system in tolerance seems to be recovered in abstinent rats and might be part of the molecular mechanisms underlying cannabinoid dependence (Rubino *et al*., 1998).

Taken together, the antagonist-precipitated withdrawal seems to unmask the development of an underlying neuroadaptive process that contribute to the development of cannabinoid dependence. The recent studies provide convincing evidence that Δ^9-THC as well as other psychotropic cannabinoids are capable of producing physical dependence. Further experiments are expected to identify the neuronal systems which subserve the syndrome of cannabinoid withdrawal.

A common feature of withdrawal from drugs of abuse is a negative affective state that is characterized by dysphoria and anxiety and in animals by a reward deficit and enhanced reactivity to stressors (Koob, 1996). Rodriguez de Fonseca *et al*. (1997) have shown that cannabinoid withdrawal, induced by SR141716A following long-term cannabinoid exposure, results not only in enhanced behavioral responses to stressors but also in an increased release of corticotropin releasing factor (CRF) and was associated with an induction of c-fos in the limbic system as well as in the ventral tegemental area, locus coeruleus, central gray and area postrema of the rat (Rodriguez de Fonseca *et al*., 1997). Maximal increases in CRF correspond to the time when behavioral symptoms of cannabinoid withdrawal were at a maximum. Furthermore, the authors showed that the cannabinoid antagonist SR141716A did not alter the release of CRF in cannabinoid-naive rats. Thus, the neurobiological consequences of cannabinoid withdrawal, in particular the elevation of CRF release are similar to those observed during withdrawal from ethanol, cocaine and opiates (Koob, 1996; Rodriguez de Fonseca *et al*., 1997). Therefore, these authors have suggested that, due to the neuroadaptive changes, chronic cannabinoid exposure may provide a neurobiological basis for the gateway hypothesis. This means, by the activation of CRF function, cannabinoid abuse could lead to a subtle disruption of hedonic systems in the brain that are

then primed for further disruption by other drugs of abuse.

Previously, the toxicity of marijuana smoking was considered to be low. However, it should be emphasized that it may produce a toxic encephalopathy in young humans [for review see Court (1998)], when also it does not cause irreversible cerebral damage. It seems to be generally accepted that chronic cannabinoid exposure (at least for several months) can produce short- and long-term cognitive impairments as well as a reduced ability to focus attention and to filter out irrelevant information (Solowij *et al.*, 1995). Unlike ethanol, impairment may not be so clearly dose-dependent. Furthermore, chronic exposure to cannabis may cause long-term impairment. It has been reported that residual neuropsychological effects, as evidenced by greater cognitive impairments, persist even after abstinence (Pope and Yurgelun-Todd, 1996). However, the question remains open as to whether this impairment is due to a residue of drug in the brain, a withdrawal effect from the drug, or a direct neurotoxic effect of Δ^9-THC. Chan *et al.* (1998) have just presented ample evidence for the latter possibility, that is, Δ^9-THC-induced neurotoxicity. Following treatment of cultured hippocampal neurons or hippocampal slices with Δ^9-THC, they observed shrinkage of neuronal cell bodies and nuclei as well as fragmentation of DNA, indicating neuronal apoptosis (see also Section 5.3). These effects occurred at Δ^9-THC concentrations as low as 0.5–1 μM, which are comparable to the Δ^9-THC levels measured in human plasma after consumption of marijuana. The neuronal cell death is assumed to be involved in the deficiencies in memory after long-lasting consum of marijuana. In addition to these neurotoxic effects of Δ^9-THC, smoking marijuana exposes users to 50% higher levels of procarcinogen benz-α-pyrene than does smoking tobacco (Hoffman *et al.*, 1975), and to carboxyhemoglobin levels and tar levels that are five times higher and three times higher than those produced by tobacco smoking, respectively (Wu *et al.*, 1983).

3. CANNABINOID RECEPTORS

Since the major psychoactive constituent of marijuana, Δ^9-THC, as well as other cannabinoids are highly lipophilic molecules, it was believed for a long time that the effects elicited by these compounds are due to a non-specific interaction with the membrane lipids and alteration of membrane fluidity. Speculation about the cellular actions of cannabinoids was finally resolved when functional inhibition of adenylate cyclase was observed following the addition of Δ^9-THC to neuroblastoma cells (Howlett and Fleming, 1984). This action as well as studies demonstrating stereoselectivity of the (–)-enantiomers of Δ^9-THC (Dewey, 1986) and specific binding of radiolabeled agonists in rat brain membranes (Devane *et al.*, 1988) indicated that most of the central cannabinoid effects were mediated by a specific membrane receptor protein, the CB1 receptor. Till now, two cannabinoid receptors, the CB1 and the CB2 receptor have been described with regard to their primary structure, ligand-binding properties, and signal transduction systems. Complementary DNAs for both cannabinoid receptors have been cloned, and the expression of their genes and the functional domains within the proteins have been investigated. A splice variant of the CB1 receptor has also been isolated and has been designated CB1A (Shire *et al.*, 1995). The distribution of the cannabinoid receptors has been mapped using various techniques, including receptor binding and autoradiography, northern blot techniques, *in situ* hybridization, an reverse-transcription polymerase chain reaction. Recently, localization using an antibody to the CB1 receptor (Sinha *et al.*, 1998) and to the CB2 receptor (Galiègue *et al.*, 1995) has been described. Initially, it was believed that the CB1 receptor was localized exclusively in the brain and testis, whereas the CB2 receptor was localized peripherally in cells and tissues derived from the immune system. However, it is gradually emerging that this distinction is not so simplistic.

3.1. The Cannabinoid CB1 Receptor

3.1.1. *Distribution and Properties of the CB1 Receptor*

The CB1 receptor is one of the most abundantly expressed of the neuronal receptors. Its distribution has been well characterized in rat (Herkenham *et al.*, 1990, 1991a,b; Mailleux and Vanderhaeghen, 1992; Tsou *et al.*, 1998) and human (Westlake *et al.*, 1994; Glass *et al.*, 1997) brain. Specific, saturable, high affinity CB1 binding sites exhibit a widespread distribution in the brain and correlates well with the effects of cannabinoids on memory, perception, and the control of movement. In autoradiographic studies using a radioactive cannabinoid, it has been shown that within the brain, these binding sites are not distributed homogeneously (Herkenham *et al.*, 1990, 1991a,b; Mailleux and Vanderhaeghen, 1992). In many brain areas, CB1 receptor density greatly exceeds that of neuropeptide receptors and is similar to the densities of cortical benzodiazepine, striatal dopamine and even whole-brain glutamate receptors (Herkenham *et al.*, 1990). The highest density has been demonstrated in the basal ganglia (substantia nigra, globus pallidus, enteropeduncular nucleus and lateral caudate putamen) and the molecular layer of the cerebellum. The high density of cannabinoid receptors in the basal ganglia is consistent with the marked effects that cannabinoids exert on spontaneous locomotor activity in rodents, for instance, ataxia during acute intoxication (Compton *et al.*, 1991; Martin *et al.*, 1991, 1998; Pertwee, 1993) (see also Section 5.1). It is intriguing that in human brain, receptor expression in the cerebellum is substantially reduced in comparison with rats (Herkenham *et al.*, 1991b). A lower abundance of cannabinoid receptors in the cerebellum is in line with the absence of gross motor disturbances experienced by human exposed to marijuana.

High binding densities were also found in the CA pyramidal cell layers of the hippocampus, the dentate gyrus and layers I and IV of the cortex. The high densities in these brain regions provide a basis

for understanding previous pharmacological data demonstrating an involvement of cannabinoids in the impairment of cognition and memory (see also Section 5.3). Furthermore, they may explain the anticonvulsant effects of these compounds. Intermediate levels of binding sites were found in the nucleus accumbens which is associated with mediating brain reward. Binding is sparse or absent from areas of brain stem, medulla oblongata and hypothalamus. Since these regions control cardiovascular and respiratory functions, the low binding densities may explain the relative low acute toxicity and lack of lethality of marijuana. The brain distribution of cannabinoid binding sites has been studied not only autoradiographically, but also by performing saturation binding assays with homogenated tissue from various areas of rat brain, using a radiolabeled agonist (Kuster *et al*., 1993). These studies revealed that the K_d values of the cannabinoids do not vary between the brain areas. Recently, the immunohistochemical distribution of the CB1 receptor has been studied in the adult rat brain (Tsou *et al*., 1998). These results supported the findings of the autoradiographic studies. However, because of the greater resolution obtained with the immunohistochemical study, intriguing new data have been presented about the identification of particular neuronal cell fibers that possess CB1 receptors. The authors have observed CB1 receptor-like immunoreactivity in axons, cell bodies and dendrites, where it appeared as puncta in somata and processes. Intensely stained neurons were dispersed and only occurred in cortical structures including hippocampal formation and olfactory bulb. In the hippocampal formation, the highest density of stained neurons has been observed. The cell bodies of pyramidal neurons in CA1 and CA3 fields appeared to be unstained but surrounded by a dense plexus of immunoreactive fibers. Moderately or lightly stained neurons occurred in caudate-putamen and amygdala and beaded immunoreactive fibers have been detected in periaqueductal gray as well as in the dorsal horn and lamina X of the spinal cord (Tsou *et al*., 1998). The periaqueductal gray, and the spinal cord, in turn, are important sites in ascending pain transmission (Behbehani, 1995), and the CB1 receptor in these regions are expected to be involved in the antinociception induced by cannabinoids (see Section 2.2.2). While the CB1 receptor is widely distributed in the forebrain, it has a more restricted distribution in the hindbrain and the spinal cord.

Some open questions raises regarding the distribution of the CB1 receptor. Thus, there are inconsistencies in the distribution pattern of the receptor and the pharmacological action of its agonists. For instance, certain areas of the CNS which are known to be cannabinoid-sensitive are quite sparsely populated with CB1 receptors. These include the hypothalamus, at which cannabinoids are thought to bring about hypothermia, as well as the brain stem and spinal cord, both putative sites for the cannabinoid-induced antinociception.

Although it has been assumed in the past that CB1 receptors occur exclusively in the brain, there is evidence for a peripheral localization, too. In the periphery, CB1 receptors have been found in spleen and tonsils, and at very low levels also in adrenal gland, heart, prostata, uterus, ovary (Galiègue *et al*., 1995), testis (Gerard *et al*., 1991; Galiègue *et al*., 1995), and presynaptically on sympathetic nerve terminals (Ishac *et al*., 1996). The activation of presynaptic CB1 receptors located on peripheral sympathetic nerve terminals is presumed to mediate sympathoinhibitory effects *in vitro* and *in vivo*.

The cannabinoid CB1 receptor cDNA was isolated firstly from a rat cerebral cortex library by an oligonucleotide probe derived from a member of G protein-coupled receptors (Matsuda *et al*., 1990). Chinese hamster ovary cells that were stably transfected with this cDNA became responsive to cannabinoids in assays for cAMP accumulation, demonstrating cannabinoid-mediated inhibition of adenylate cyclase (Matsuda *et al*., 1990). Shortly thereafter, the cloning of a human CB1 receptor cDNA was reported (Gerard *et al*., 1991). The rat and human receptors are highly conserved, with 93% identity at the nucleic acid level and 97% homology at the amino acid level. The gene locus for the human CB1 receptor has been genetically mapped to chromosome 6 based on a restriction fragment length polymorphism (Caenazzo *et al*., 1991). Physical mapping by *in situ* hybridization to metaphase chromosomes has confirmed this location and has further defined the locus to position 6q14-q15 (Hoehe *et al*., 1991). The mouse CB1 receptor gene is located on chromosome 4 (Onaivi *et al*., 1996).

Analysis of the primary amino acid sequence of the CB1 receptor predicts the presence of seven hydrophobic transmembrane domain regions which extend through the plasma membrane, intervening loops extending extra- and intracellularly as well as an extracellular N-terminus and an intracellular C-terminus. With regard to its structure, the cannabinoid CB1 receptor exhibits the basic structural features predicted for a G protein-coupled receptor. With use of an antipeptide antibody in immunoblots, the apparent molecular weight of the CB1 receptor is 64 kDa (Song and Howlett, 1995). Treatment of this receptor with endoglycosidases F and/or H reduces the apparent weight of the receptor to 59 and 53 kDa, indicating that it is glycosylated. Indeed, the primary structure of the CB1 receptor contains the consensus sequence for three potential glycosylation sites on the N-terminal extracellular extension (Matsuda *et al*., 1990; Song and Howlett, 1995). N-linked glycosylation is a posttranslational modification occurring as a series of enzymatic reactions beginning in the rough endoplasmatic reticulum and continuing through the Golgi apparatus prior to the translocation of receptor proteins to the plasma membrane.

Many members of the G protein-coupled receptor superfamily contain conserved cysteine residues that appear to stabilize the tertiary structure of the receptor due to their involvement in an intramolecular disulfide bridge. In most receptors, these cysteines occur in the extracellular domains that lie between the second and third transmembrane domain (i.e. the second extracellular loop) as well as between the forth and fifth hydrophobic domains (i.e. the third extracellular loop). In the CB1 receptor, however, no cysteines are found within the second extracellu-

lar loop, but the third extracellular loop contains two or more cysteines (Matsuda, 1997). A further difference to most other G protein-coupled receptors is that CB1 receptors lack a highly conserved proline residue in the fifth hydrophobic domain. Recently, Mahmoudian (1997) developed a three-dimensional model of the human cannabinoid CB1 receptor by use of computer-aided molecular modeling techniques. According to this model, there may be a calcium-binding site in the extracellular N-terminal domain. The ligand binding site is predicted to be localized at a hydrophobic site. The phenolic hydroxyl group of the ligand seems to form a hydrogen bond with the carboxy group of Ala-198 at the receptor protein (Mahmoudian, 1997).

Shire *et al.* (1995) have isolated a splice variant of the cannabinoid CB1 receptor from a human lung cDNA library. The sequence of this splice variant (CB1A) revealed a deletion of 167 base pairs near the 5′ end of the coding region that occurs between consensus splice donor and acceptor sequences in the CB1 receptor sequence. The alternatively spliced receptor RNA would encode a NH_2-terminal modified isoform (CB1A) of the CB1 receptor, shorter than CB1 by 61 amino acids with a molecular weight of ca 46 kDa. While the majority of the primary sequence of the CB1A variant is identical to that of the CB1 receptor, the first 28 amino acids of the N-terminal of the putative truncated receptor are completely different from those of the CB1 receptor (Caput and Ferrara, 1995). In this part, the CB1A receptor contains more hydrophobic residues. N-Linked glycosylation of the CB1A receptor protein is unlikely, because the consensus sites found in the N-terminal domain of the CB1 receptor are absent in the truncated splice variant. The alternative splice form is unusual in that it is generated from the mRNA encoding CB1, and not from a separate exon (Shire *et al.*, 1995). Rat CB1 mRNA seems to be similarly alternatively spliced. A study of the distribution of the human CB1 and CB1A mRNAs by reverse transcription-polymerase chain reaction analysis showed the presence of both CB1 and CB1A throughout the brain but also in the periphery (Shire *et al.*, 1995). In general, the authors detected CB1A receptor mRNA in all tissues where CB1 receptor mRNA has been found. The relative quantities of the mRNAs encoding these two receptors are rather uniform in peripheral tissues. The CB1A receptor mRNA is present in amounts of ca 10% of the CB1 receptor mRNA. In the brain, however, the relative quantities of receptor mRNAs are more varied, the levels of CB1A receptor mRNA being <1–20% of the levels of CB1 receptor mRNA (Shire *et al.*, 1995). Both cannabinoid receptor agonists and antagonists bind also to the CB1A receptor, however, with somewhat lower affinity (Rinaldi-Carmona *et al.*, 1996). A functional comparison of the CB1 and CB1A receptors has not yet been published.

The expression of the CB1 receptor mRNA can be induced or repressed by various factors. In rat brain, an increase in CB1 receptor RNA as been observed following the manipulation of either the glucocorticoid hormone or dopaminergic neurotransmitter systems. In adrenalectomized rats, CB1 receptor mRNA levels in the striatum increased by ca 50% (Mailleux and Vanderhaeghen, 1993a). If these animals also received treatment with dexamethosone, the increase was counteracted and CB1 receptor mRNA was reduced by 30%. These findings suggests that glucocorticoids can downregulate cannabinoid receptor gene expression. Similarly, in rats that received unilateral 6-hydroxydopamine dopaminergic deafferentation of the striatum, CB1 receptor mRNA is elevated in the ipsilateral side by ca 45% (Mailleux and Vanderhaeghen, 1993b). Furthermore, treatment with dopamine receptor antagonists mimicked this effect. Like glucocorticoids, dopaminergic activity seems to reduce the expression of CB1 receptor mRNA in the striatum. In contrast, CB1 receptor mRNA levels seems to be decreased following surgical or pharmacological treatment that disrupts glutamatergic innervation. Following unilateral cerebral decortication or pharmacological treatment with the NMDA receptor antagonist MK-801, mRNA levels were reduced by ca 30 and 52% in the ipsilateral nucleus caudatus, respectively, compared with the intact side of the brain (Mailleux and Vanderhaeghen, 1994). These findings imply a NMDA receptor-mediated upregulation of cannabinoid receptor mRNA levels. The mechanisms involved (transcriptional or posttranscriptional) in the glucocorticoid, dopaminergic and glutamatergic regulation of CB1 receptor mRNA are still unknown.

3.1.2. *Signal Transduction Mechanisms*

Cannabinoid CB1 receptors couple to multiple signal transduction pathways including adenylate cyclase, mitogen-activated protein (MAP) kinase and ion channels.

3.1.2.1. *Inhibition of adenylate cyclase*

Already prior to the characterization of a specific membrane receptor, experiments performed with neuroblastoma cells, rat brain slices and cultured cerebellar neurons demonstrated that Δ^9-THC and other cannabinoids are capable of inhibiting adenylate cyclase in a reversible, dose-dependent, and stereoselective manner (Howlett and Fleming, 1984; Howlett *et al.*, 1986; Bidaut-Russell *et al.*, 1990; Pacheco *et al.*, 1993). This discovery was the basis of the concept that the cannabinoid actions are mediated by G protein-coupled membrane receptors. In neuroblastoma cells, maximal inhibition of adenylate cyclase occurred at a Δ^9-THC concentration of 1 μM or less (Howlett and Fleming, 1984). There is clear evidence that the CB1 receptor is involved in the cannabinoid-induced attenuation of cAMP production. Firstly, an inhibition of adenylate cyclase is not observed in either the soluble adenylate cyclase from rat sperm or in not membrane-bound adenylate cyclases preparations from different cell lines. This cellular selectivity provides further evidence for the existence of specific receptors for the cannabimimetic compounds. Secondly, this is in line with the finding that brain regions in which cannabinoids are most effective inhibitors of adenylate cyclase are those with the highest density of CB1 receptors, that is, hippocampus, striatum,

cerebral cortex and cerebellum (Bidaut-Russell *et al.*, 1990). Thirdly, it has been demonstrated in experiments with Chinese hamster ovary cells transfected with human CB1 receptors that the cannabinoid-induced inhibition of cAMP production can be antagonized by the selective CB1 receptor antagonist, SR141716A (Rinaldi-Carmona *et al.*, 1994; Bouaboula *et al.*, 1995a; Felder *et al.*, 1995).

The involvement of an inhibitory G protein has been clearly demonstrated, because the cannabinoid-induced inhibition of the adenylate cyclase can be inhibited by pertussis toxin in mammalian brain as well as in cultured neuronal cells (Howlett *et al.*, 1986; Bidaut-Russell *et al.*, 1990; Pacheco *et al.*, 1993; Bouaboula *et al.*, 1995a). This is a strong indication that cannabinoid receptors are indeed negatively coupled to adenylate cyclase through $G_{i/o}$ proteins, because pertussis toxin prevents the dissociation of the α and β/γ subunits of such G proteins by covalent ADP-ribosylation, thereby blocking the G protein-mediated inhibition of adenylate cyclase. Moreover, the cannabinoid regulation of adenylate cyclase is sensitive to divalent cations and guanine nucleotides in a manner characteristic for other G_i protein-coupled receptors. The inhibition was greatest at micromolar concentrations of Mg^{2+} or Mn^{2+} (Howlett *et al.*, 1991). In membranes prepared from neuroblastoma cells or rat brain, inhibition of adenylate cyclase by cannabinoids requires the addition of supplemental GTP (Howlett, 1985; Childers *et al.*, 1994). Sim *et al.* (1995) have localized CB1 receptor-mediated activation of G proteins by receptor-stimulated [^{35}S]GTPγS binding by *in vitro* autoradiography. This study revealed that the anatomical distribution of agonist-stimulated [^{35}S]GTPγS binding qualitatively paralleled receptor distribution as determined by receptor binding autoradiography. The most important result of this study was that all cannabinoid receptors detected in the brain with radioligand binding activate G proteins. However, quantitative differences suggest that variations in coupling efficiency may exist between different receptors in various brain regions (Sim *et al.*, 1995; Childers and Deadwyler, 1996).

Cannabinoid-induced inhibition of adenylate cyclase is not mediated by non-cannabinoid receptors which are also known to be negatively coupled to this enzyme by G proteins. It has been demonstrated that interaction of cannabinoid compounds with α_2-adrenergic, M_4-muscarinic, or δ-opioid receptor did not occur because antagonists of those receptors failed to block the Δ^9-THC-induced effect in neuroblastoma cells (Howlett and Fleming, 1984) and in rat cerebellar membranes (Pacheco *et al.*, 1993). However, the cannabinoid CB1 receptor is known to share G proteins with other G protein-coupled receptors, as demonstrated by additivity experiments in cultured cerebellar granule cells (Devane *et al.*, 1986; Childers *et al.*, 1992; Pacheco *et al.*, 1993) and striatal slices (Bidaut-Russell and Howlett, 1991). Agonists of different $G_{i/o}$ protein-coupled receptor systems were added alone or in combination, to determine whether any portion of the signal transduction pathway is shared. The maximal inhibition of the cAMP accumulation obtained with cannabinoids was shown in these studies to be not increased by addition of maximally effective concentrations of agonists of the muscarine receptor, α_2-adrenergic receptor, dopamine D_2 receptor, $GABA_B$ and opioid receptor. These studies have two implications: first, the CB1 receptor seems to be colocalized with other G protein-coupled receptor on the same neuron, and second, they must share a common coupling mechanism. However, in contrast to the lack of additivity in inhibition of adenylate cyclase, $GABA_B$ agonists and cannabinoid agonists produce a full additive response in stimulating low-K_m GTPase activity in cerebellar granule cells (Pacheco *et al.*, 1993). This finding suggest that different pools of G proteins are stimulated by different receptors but that they share a common effector system, that is, adenylate cyclase (Fig. 2).

As reported in Section 2.3, recent results indicate that G proteins are also involved in the development of tolerance to cannabinoids. Rubino *et al.* (1998) have shown by in situ hybridisation that besides downregulation of the CB1 receptor a reduction of $G\alpha_i$ expression occurs in the forebrain, cerebellum and mesencephalon of cannabinoid-tolerant rats, suggestive for an alteration of G protein expression. This can be a molecular event associated with the development of cannabinoid tolerance.

3.1.2.2. *Modulation of ion channels*

$G_{i/o}$ proteins not only couple various receptors to adenylate cyclase, thereby mediating an attenuation of cAMP production, but also to ion channels. The CB1 receptor has been reported to modulate the activity of N- and P/Q-type voltage-dependent calcium

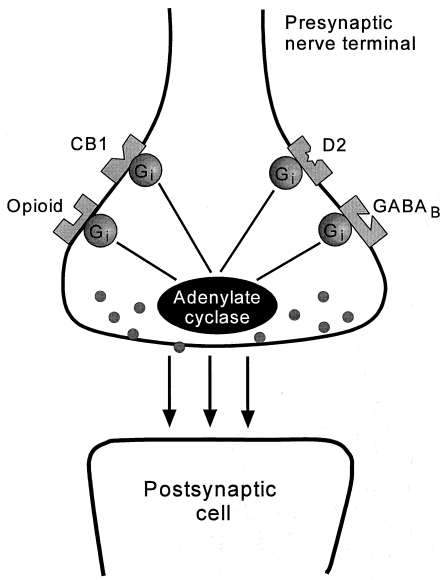

Fig. 2. Schematic diagram illustrating the possible interaction of the cannabinoid CB1 receptor with other $G_{i/o}$-coupled receptors, such as opioid receptors, the dopamine D2 receptor and the $GABA_B$ receptor. These receptors stimulate an own pool of G proteins, but share a common adenylate cyclase [modified from Childers and Deadwyler (1996)].

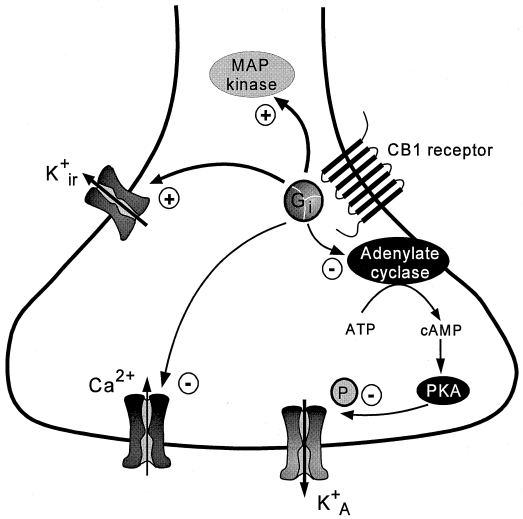

Fig. 3. Signal transduction mechanisms stimulated by the cannabinoid CB1 receptor in a presynaptic nerve terminal. Activation of the CB1 receptor stimulates the coupling to a pertussis toxin-sensititve G protein. The G protein transduces the cannabimimetic stimulation of mitogen-activated protein kinase (MAP kinase) and the inhibition of adenylate cyclase, thereby attenuating the production of cAMP. Moreover, the G protein directly couples the CB1 receptor to N- and Q/P-type voltage-dependent calcium channels, which are inhibited, and to inwardly rectifying potassium channels (K_{ir}^+), which are stimulated by cannabinoids. The activation of the A-type potassium current (K_A^+) is also G protein-dependent, but it is modulated via the cannabinoid-induced inhibition of adenylate cyclase. Due to the decrease in cAMP accumulation, cAMP-dependent protein kinase (PKA) is inhibited by CB1 receptor activation. In absence of cannabinoids, the cAMP-dependent protein kinase phoshorylates the A-type potassium channel protein, thereby exerting a negative shift in its voltage-dependence. In presence of cannabinoids, however, the phoshorylation of the channel by cAMP-dependent protein kinase is reduced. This leads to a positive shift in voltage-dependence and to an enhanced outward potassium current. Receptor-effector coupling which is enhanced by cannabinoids is marked by thicker lines, those which are inhibited are marked by thinner lines.

channels and to enhance the activation of the voltage-dependent A-type potassium channel and the inwardly rectifying potassium channel (Fig. 3).

It was reported previously that cannabinoids exert an inhibitory effect on voltage-activated inward calcium currents in various neuroblastoma cell lines (Caulfield and Brown, 1992; Mackie and Hille, 1992) and primary neurons expressing rat brain cannabinoid CB1 receptors (Pan *et al.*, 1996). This effect is slow in onset (minutes) and recovery (minutes to hours) as well as pertussis toxin-sensitive indicating a $G_{i/o}$ protein-mediated process. Cannabinoids seem to affect the N-type and the P/Q-type calcium channel, because inhibition of calcium currents by Δ^9-THC and other cannabinoids is attenuated by the selective N-type calcium channel blocker ω-conotoxin GVIA and by the selective P/Q-type blocker ω-agatoxin. In contrast, L-type calcium channel blockers do not change the cannabinoid-induced inhibition of the calcium inward current (Caulfield and Brown, 1992; Mackie and Hille, 1992). There is strong evidence that the cannabinoid-induced decrease in the calcium inward current is not mediated by the inhibition of adenylate cyclase. The inhibitory effect of cannabinoids on calcium currents has been reported to persist in neuroblastoma cells despite the presence of stable analogs of cAMP and of phosphodiesterase inhibitors (Mackie and Hille, 1992).

For a long time, the actions of cannabinoids on calcium currents in central neurons have been unexplored. More recently, however, electrophysiological experiments performed in the whole-cell configuration of the patch clamp technique in rat hippocampal neurons have extended the earlier work from cell lines and transfected neurons (Twitchell *et al.*, 1997; Shen and Thayer, 1998). These experiments have demonstrated that the inhibition of the N-type calcium current by cannabinoids is stereospecific, pertussis toxin-sensitive and inhibited by the selective CB1 receptor antagonist SR141716A indicating a CB1 receptor-mediated process. However, high concentrations of agonist, *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolol[1,2,3-de]-1,4-benzoxazinyl]-(1-naphtalenyl)methadone (WIN55 212, $>1\ \mu$M), exert an inhibition of the calcium current which was not blocked by the antagonist suggesting that WIN55 212 may alter the calcium conductance also by a direct, non-receptor-mediated action on the channel (Shen and Thayer, 1998).

Taken together, these results demonstrate a CB1 receptor-mediated inhibition of N- and P/Q-type calcium currents in central neurons. Since the calcium channels that underlie these currents are chiefly located presynaptically, and are required for evoked neurotransmitter release in the CNS (Takahashi and Momiyama, 1993; Wheeler *et al.*, 1994), the results suggest a major role for cannabinoids (endogenous and exogenous) in the modulation of synaptic transmission at CNS synapses. Inhibition of presynaptic calcium channels by cannabinoids likely reduces neurotransmitter release from CB1-expressing presynaptic terminals. Indeed, cannabinoids have been shown to inhibit via CB1 receptors the release of glutamate both in cultured rat hippocampal cells (Shen *et al.*, 1996) and cerebellar Purkinje cells (Levenes *et al.*, 1998), acetylcholine release in superfused rat hippocampal slices (Gifford *et al.*, 1997), and noradrenaline release in slices from human, guinea-pig and rat hippocampus (Schlicker *et al.*, 1997). Thus, the presynaptic inhibition of several neurotransmitters by cannabinoids may turn out to be a key neuronal effect of cannabinoids.

The presynaptic inhibition of neurotransmitter release by cannabinoids seems to be highly effective due to two facts: First, cannabinoid CB1 receptors appear to be present at higher density at the presynaptic terminal relative to the soma (Herkenham *et al.*, 1990, 1991b; Twitchell *et al.*, 1997). Second, the activation of potassium channels by cannabinoids (see below) can amplify a presynaptic inhibition of calcium channels by reducing the duration of the action potential. Since the relationship between presynaptic calcium influx and neurotransmitter release is expressed by a high power law (Mintz *et al.*, 1995), it is concluded that the cannabinoid-induced inhibition of transmitter release is greatly amplified.

This means, small decreases in presynaptic calcium influx produce large decreases in transmitter release. These effects might represent a plausible cellular mechanism underlying the well-known psychoactive actions but also the antinociceptive action caused by cannabinoids.

As already mentioned above, cannabinoids are reported to modulate two potassium channels, the inwardly rectifying potassium channel and the voltage-dependent A-type potassium channel. Cannabinoids have been shown to enhance the inwardly rectifying potassium current in murine tumor cells and in Xenopus oocytes expressing CB1 receptors in a stereoselective and pertussis toxin-sensitive manner (Henry and Chavkin, 1995; Mackie *et al*., 1995) indicating a $G_{i/o}$ protein-mediated process.

A recent report has provided intriguing evidence that the ability of cannabinoids to enhance the inwardly rectifying potassium current as well as to reduce calcium currents is dependent on the phosphorylation status of the cannabinoid CB1 receptor. Garcia *et al*. (1998) have just reported that the CB1 receptor can be phosphorylated by protein kinase C. Activation of protein kinase C by phorbol esters leads to a reduced ability of cannabinoids to activate inwardly rectifying potassium currents and to attenuate P/Q-type calcium currents in a cell line transfected with rat CB1 receptors. Furthermore, the authors demonstrated that protein kinase C phosphorylated a single serine (S 317) of a fusion protein incorporating the third intracellular loop of the CB1 receptor. Exchange of this serine to alanine did not affect the ability of the CB1 receptor to modulate potassium and calcium currents, but eliminated the action of phorbol esters, demonstrating that protein kinase C can disrupt ion channel modulation by receptor phosphorylation.

Moreover, Deadwyler *et al*. (1993) have shown that the voltage dependence of the rapidly inactivating potassium A current, characteristic of hippocampal neurons, was significantly altered in a concentration-dependent manner by cannabinoid analogs. Decreased inactivation, which led to an increased activation of this current near resting levels in these cells, was observed after brief applications of cannabinoids. These actions were blocked by pertussis toxin. Cellular dialysis of GTPγS mimicked the actions of cannabinoids while blocking further effects due to added cannabinoids. These data seem to support the conclusion that cannabinoid effects on the A-type potassium current are mediated through G protein-coupled receptors. However, more recent evidence suggest that the cannabinoid-induced stimulation of this potassium current is cAMP dependent and results from CB1 receptor-mediated inhibition of adenylate cyclase and subsequent inhibition of protein phosphorylation of the A-type potassium channel protein (Fig. 3), but not through a direct coupling of G_i proteins to the A-type potassium channel (Hampson *et al*., 1995; Childers and Deadwyler, 1996). This is supported by the findings that similar activation on the A-type potassium currents have been produced by the non-hydrolyzable GTP analog, GTPγS and inhibitors of protein kinase A, whereas an inhibition of the current was elicited by the membrane-permeable analog of cAMP, 8-bromo-cAMP, by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) and by stimulation of adenylate cyclase with forskolin (Deadwyler *et al*., 1993; Hampson *et al*., 1995; Childers and Deadwyler, 1996).

3.1.2.3. *Activation of MAP kinase*

A third well characterized messenger system of the CB1 receptor is the stimulation of MAP kinase (Bouaboula *et al*., 1995b). Recently, the importance of the MAP kinase family for the mediation of morphological differentiation and promotion of survival in neurons has been extensively reviewed (Fukunaga and Miyamoto, 1998). Several members of this family are abundant in the CNS and are activated during various physiological and pathological events such as brain ischemia and epilepsy.

Thus far, the activation of the MAP kinase pathway by the CB1 receptor has only been found in cell lines. Therefore, it is still uncertain whether it plays a significant physiological role in the CNS. Using stably transfected Chinese hamster ovary cells expressing human CB1 receptors it has been demonstrated that cannabinoids are highly potent activators of MAP kinase phosphorylation (Bouaboula *et al*., 1995b). Inhibition of this effect by the selective antagonist, SR141716A, implying an involvement of the CB1 receptor. This is further supported by the finding that in untransfected Chinese hamster ovary cells cannabinoids do not activated MAP kinase. Moreover, the activation of MAP kinase has been shown to be blocked by pertussis toxin pointing to an involvement of $G_{i/o}$ proteins. Since cAMP can prevent the activation MAP kinase, and since the CB1 receptor mediates an attenuation of cAMP production, the possibility arises that the cannabinoid-induced activation of MAP kinase appear subsequent to the decreased intracellular cAMP level. However, treatment of the transfected Chinese hamster ovary cells with hydrolysis-resistant cyclic AMP analogues or with the phosphodiesterase inhibitor, IBMX, failed to activate MAP kinase (Bouaboula *et al*., 1995b). This finding suggests that the signal transduction pathway between the CB1 receptor and the MAP kinase involves a pertussis toxin-sensitive G protein and is independent of the cannabinoid-induced inhibition of cAMP production (Bouaboula *et al*., 1995b). This coupling of the CB1 receptor to a mitogenic signal pathway may be an intermediate step in the cannabinoid receptor-induced expression of the immediate early gene *Krox*-24 (Bouaboula *et al*., 1995b).

3.2. The Cannabinoid CB2 Receptor

The CB2 receptor is restricted to the periphery and has not been detected in the central nervous system (Munro *et al*., 1993; Galiègue *et al*., 1995). Although the present review will focus on the cannabinoid-induced effects on the brain, the pharmacology of the CB2 receptor will be briefly presented, in order to compare both receptor types and to understand the peripherical side-effects of marijuana and cannabimimetic drugs. Table 1 summarizes the

Table 1. Comparison of the cannabinoid CB1 and CB2 receptor

	CB1	CB2
Molecular weight	53 kDa	40 kDa
Localization	Brain≫spleen and tonsils	Various peripheral tissues (spleen > tonsils > immune cells)
Signal transduction	Adenylate cyclase ↓ MAP kinase ↑ K_{ir}^+ ↑ K_A^+ ↑ Ca^{2+} ↓ (N- and P/Q-type)	Adenylate cyclase ↓ MAP kinase ↑
Agonists	Δ^9-THC > anandamide > cannabinol > cannabidiol	Δ^9-THC > cannabinol > cannabidiol > anandamide
Antagonists	SR141716 LY320135	SR144528

differences between the cannabinoid CB1 and CB2 receptor.

3.2.1. *Distribution and Properties of the CB2 Receptor*

The second cannabinoid-binding receptor, the CB2 receptor, was isolated by its homology to other G protein-coupled receptors, using a polymerase chain reaction assay in myeloid cells (Munro *et al.*, 1993). This receptor type shares with the CB1 receptor the structural feature typified by seven transmembrane spanning domains and is coupled to a pertussis toxin sensitive G protein. The human CB2 receptor clone was derived from a human promyelocytic leukemia cell HL60 cDNA library. The clone has 68% amino acid sequence identity to the CB1 receptor within the transmembrane domains and only 44% identity throughout the total protein (Munro *et al.*, 1993). There are considerable differences in the size of the CB1 and the CB2 receptor (Table 1). While the human CB1 receptor consists of 472 amino acids, the human CB2 receptor consists of only 360 amino acids and has molecular weight of ca 40 kDa (Matsuda, 1997). This discrepancy seems to refer to the difference in length of the extracellular N-terminal domain. In this part of the protein molecule, the CB2 receptor lacks 83 amino acid compared with the CB1 receptor.

The localization of the CB2 receptor differs markedly from that of the CB1 receptor. The occurrence of the CB2 receptor is restricted to the periphery where it has been found in the marginal zone of the spleen (Munro *et al.*, 1993; Schatz *et al.*, 1997), in tonsils and immune cells (B-cells, monocytes, T-cells) (Munro *et al.*, 1993; Galiègue *et al.*, 1995; Schatz *et al.*, 1997). In spleen and tonsils, the CB2 mRNA content has been reported to be equivalent to that of CB1 mRNA in the brain (Galiègue *et al.*, 1995). Among the main blood cell subpopulations, the distribution of CB2 receptors displayed important differences, with a much higher level in B cells than in monocytes and T cells. The localization of CB2 receptors in immune tissues is responsible for the immunosuppressive properties of marijuana reported in Section 2.2.4. These findings imply that the cannabinoid-induced immunosuppression involves a receptor-mediated process. Moreover, although CB1 receptors also may occur in the immune system, there is evidence that immunosuppression is mediated mainly the CB2 receptor, since the CB1 mRNA levels represent only 1–10% of the CB2 content (Galiègue *et al.*, 1995).

The ligand selectivities of both the CB1 and the CB2 receptors are rather similar. However, the endogenous cannabinoid, anandamide, has been reported to have a marginally greater affinity for the CB1 than for the CB2 receptors, whereas cannabinol has a has a greater affinity for the CB2 receptor (Matsuda, 1997).

3.2.2. *Signal Transduction Mechanisms*

As might be already expected with regard to the striking dissimilarities in sequence homology of the CB1 and the CB2 receptor protein, both cannabinoid receptors show also differences in their signal-effector coupling (Table 1). Thus, in marked contrast to the CB1 receptor, activation of the CB2 receptor has not yet been shown to affect ion channels. Felder *et al.* (1995) failed to observe a detectable effect of cannabinoids on calcium currents and on the inwardly rectifying potassium current in Chinese hamster ovary cells expressing CB2 receptors. However, as predicted by the seven transmembrane domains, the CB2 receptor is also coupled to a G protein, thereby negatively coupled to adenylate cyclase.

3.2.2.1. *Inhibition of adenylate cyclase*

The evidence that the CB2 receptor is negatively coupled to adenylate cyclase became obvious by the finding that cannabinoids inhibit concentration-dependently forskolin-induced cAMP production in Chinese ovary hamster cells transfected with CB2 mRNA (Slipetz *et al.*, 1995). In contrast, they failed to inhibit cAMP accumulation in non-transfected cells. The CB2 receptor-mediated inhibition of forskolin-induced cAMP production was abolished by pretreatment of the cells with pertussis toxin. These results imply that the CB2 receptor is functionally coupled to inhibition of adenylyl cyclase activity via a pertussis toxin-sensitive $G_{i/o}$ protein (Slipetz *et al.*, 1995). The CB1 receptor antagonist, SR141716A, does not prevent the attenuation of cAMP production by Chinese ovary hamster cells transfected with CB2 receptors (Rinaldi-Carmona *et al.*, 1994). In contrast, the recently discovered CB2 receptor antagonist, *N*-[(1*S*)-endo-1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-

1(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528), antagonizes the inhibitory effects of cannabinoids on forskolin-stimulated adenylyl cyclase activity in cell lines permanently expressing the human CB2 receptor ($EC_{50} = 10$ nM) but not in cells expressing the CB1 receptor (no effect at 10 μM) (Rinaldi-Carmona *et al*., 1998). Moreover, these authors have shown that SR144528 antagonizes the stimulating effects of cannabinoids on human tonsillar B-cell activation. There is further evidence that the inhibition of adenylate cyclase and, consequently, the decreased cAMP signaling that is produced by cannabinoids in leukocytes are closely correlated with decreased immune function, because the inhibitory effects produced by cannabinoids on immunocompetent cells can be abrogated by blocking or reversing the cannabinoid-induced decrease in intracellular cAMP (Herring *et al*., 1998). Taken together, these findings strongly support the hypothesis that the immunomodulatory action of cannabinoids is mediated by the CB2 receptor which negatively coupled to adenylate cyclase.

3.2.2.2. *Activation of MAP kinase*

In CB2 receptor expressing Chinese hamster ovary cells, cannabinoids have been shown to activate MAP kinase in a concentration-dependent manner (Bouaboula *et al*., 1996). Following MAP kinase activation, cannabinoids induced the expression of the growth-related immediately early gene *Krox*-24. Both the activation of MAP kinase and the induction of *Krox*-24 were abolished by pertussis toxin pretreatment, indicating the involvement of a $G_{i/o}$ protein. However, the activation of MAP kinase and the induction of *Krox*-24 were independent of an inhibition of adenylate cyclase, because membrane-permeable cAMP analogues evoke an additional enhancement of MAP kinase activation. Recently, it has been shown that SR144528 is able to selectively block the MAP kinase activity induced by cannabinoids in CB2 transfected cells (Rinaldi-Carmona *et al*., 1998), thus pointing to the involvement of the CB2 receptor. Interestingly, Bouaboula *et al*. (1996) have observed that the CB2 receptor-mediated MAP kinase activation and the *Krox*-24 induction can be attenuated by a protein kinase C inhibitor. This would suggest that, in marked contrast to the MAP kinase activation by the CB1 receptor, coupling of the peripheral CB2 receptor to a mitogenic pathway and to the regulation of *Krox*-24 expression involves protein kinase C.

3.3. Cannabinoid Receptor Ligands

3.3.1. *Agonists*

The cloning of the cannabinoid CB1 and CB2 receptor has clearly established that the most effects exerted by cannabinoid drug are receptor mediated (Matsuda *et al*., 1990). *In vitro*, at concentrations of cannabinoid agonists near the dissociation constant of the receptor, they stimulate G protein-coupled signal transduction mechanisms as described above. However at high concentrations, cannabinoid agonist stimulate the release of arachidonic acid, the inhibition of arachidonic re-acylation, and calcium release from intracellular stores (Reichman *et al*., 1991; Felder *et al*., 1992). These effects have been demonstrated to be CB1 receptor-independent in cultured cells expressing this receptor type, based on the high concentrations of cannabinoid agonists required to elicit the responses, the lack of enantiomeric selectivity, and observation of identical responses in non-transfected cells (Felder *et al*., 1992). To date, there is no clear evidence for the existence of similar CB1 receptor-independent mechanisms *in vivo*. Furthermore, it is still unknown if these effects are a result of a non-specific interaction of these lipophilic cannabinoids with membranes or proteins, or if a new receptor or receptor subtype is involved.

Availability of Δ^9-THC in its pure form and the knowledge about the primary structures of the receptor proteins provided the basis for the development of highly selective and potent receptor agonists and has contributed to advancing cannabinoid research. The synthesis of these new cannabimimetic compounds resulted from numerous structural alterations made to the basic template of Δ^9-THC and based on the three-point-attachment model of its dibenzofuran structure. The ligand binding and functional properties of CB1 and CB2 receptors have been most extensively characterized with both naturally occurring cannabinoids derived of the *Cannabis* plant and synthetic cannabimimetic compounds. The binding activities of the two cloned receptors have been studied after stable transfection into Chinese hamster ovary cells (Gerard *et al*., 1991; Bouaboula *et al*., 1996; Slipetz *et al*., 1995), COS-7 cells (Munro *et al*., 1993), and mouse AT20 cells (Felder *et al*., 1995; Mackie *et al*., 1995).

According to their chemical structure, the cannabinoid receptor agonists can be subdivided into four groups with pharmacological and behavioral similarities to Δ^9-THC. The first group consists of dibenzopyran derivatives which are represented by their prototype Δ^9-THC as well as the also in *Cannabis* plants occurring compounds Δ^8-THC, cannabinol and the non-psychoactive cannabidiol (Fig. 1).

The second group consists of bicyclic compounds represented by CP55 940 (Melvin *et al*., 1993). The potency of the compound CP55 940 (Fig. 4) is ca 4–25 times greater than of Δ^9-THC depending on the pharmacological assay. These bicyclic structures possess the minimal structural features required for analgesia and still retain most of the structural characteristics of the Δ^9-THC molecule. An extensive investigation of the structure–activity relationship revealed that maximal affinity for the CB1 receptor and maximal antinociceptive agonist activity were achieved by a C3 alkyl side chain consisting of seven or eight carbons. Side chain length of less than five carbons or more than seven or eight carbons exhibited only poor affinity for the cannabinoid receptor and were devoid of biological activity in vivo (Melvin *et al*., 1993). This indicates the importance of the lipophilic alkyl side chain at C3 of the ligand molecule for binding within a hydrophobic pocket of the receptor (Melvin *et al*., 1993). CP55 940 has been used in its tritiated form for demonstrating the distribution of CB1 receptors in the brain (Herkenham *et al*., 1990, 1991a,b).

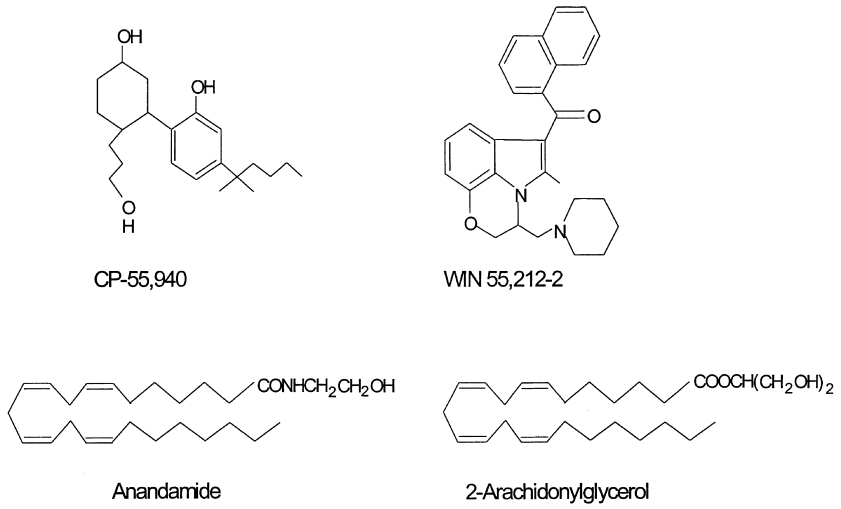

Fig. 4. Chemical structures of the synthetic cannabimimetic compounds, CP55 940 and WIN55 212-2 as well as of the endogenous cannabinoid receptor ligands, anandamide (*N*-arachidonylethanolamine) and 2-arachidonylglycerol.

The third group of cannabimimetic compounds structurally differs greatly from those occurring in the *Cannabis* plant as well as from the bicyclic cannabinoid receptor agonists. This group is made up of aminoalkylindoles and is represented by WIN55 212-2 (Fig. 4). It is intriguing that the members of this group are derivatives of parvadoline, a non-steroidal anti-inflammatory compound with analgesic opioid receptor-independent activity (Martin *et al.*, 1991; Pacheco *et al.*, 1991). The aminoalkylindoles exhibit cannabimimetic activity, which has been shown to be enantioselective, the *R*-(+)-enantiomer WIN55 212-2 being more active than the *S*(−)-enantiomer WIN55 212-3 (Pacheco *et al.*, 1991). The EC_{50} to inhibit adenylate cyclase was 0.3 and 10 μM for the *R*-(+) and the *S*(−)-enantiomer, respectively (Pacheco *et al.*, 1991). Among the cannabinoid receptor agonists, WIN55 212-2 shows a modest degree of selectivity for the CB2 receptor, whereas the other agonists are non-selective.

The fourth group of cannabinoid receptor agonists were added after the discovery of the endogenous cannabinoids (see Section 4). First, Devane *et al.* (1992) discovered cannabimimetic properties of *N*-arachidonylethanolamine (anandamide) isolated from in porcine brain, and recently, Stella *et al.* (1997) described cannabimimetic effects of 2-arachidonylglycerol isolated from rat brain. The structure of anandamide was shown to be an eicosanoid consisting of the 20 carbon fatty-acid, arachidonic acid, coupled to ethanolamine through an amide linkage (Devane *et al.*, 1992), whereas 2-arachidonylglycerol is an arachidonylester rather than an amide (Stella *et al.*, 1997). The cannabimimetic properties of anandamide and arachidonylglycerol can be predicted from their chemical structure (Fig. 4), where two of the functional groups are present, which are thought to constitute the cannabinoid pharmacophore, that is, the free hydroxyl group and the n-pentyl alkyl chain (Martin *et al.*, 1995).

There is an apparent mismatch between the structural features of the agonists required for maximal potency on one hand, and the structural features required for determining efficacy on the other hand. Sim *et al.* (1995) have demonstrated visually the relationship between an agonist (WIN55 212-2), the CB1 receptor, and a G protein by *in vitro* autoradiography of [^{35}S]GTPγS in rat brain. The WIN55 212-2-stimulated localization pattern of [^{35}S]GTPγS binding resembles the distribution of CB1 receptors as revealed by autoradiographic binding of radiolabeled agonists. However, quantitative differences in relative levels of receptor-activated G proteins compared with CB1 receptor density were observed, that is, WIN55 212-2 stimulated [^{35}S]GTPγS binding in cerebellum and cerebral cortex is lower and higher, respectively, as expected based on the binding properties (Sim *et al.*, 1995). The reason for the variability in efficacy of cannabinoid drugs in the mouse brain is unclear. More recently, Burkey *et al.* (1997) represented a quantitative determination of efficacy values for various cannabinoids measured as agonist-stimulated G protein activation in mouse brain using the [^{35}S]GTPγS assay. They have reported that the rank order of efficacy of some agonists (CP55 940 > anandamide > Δ^9-THC) does not correspond to the rank order of potency (CP55 940 ~ Δ^9-THC≫anandamide). The authors have explained this discrepancy in rank order by the fact that efficacy measures the relationship between receptor occupancy and activation of functional responses whereas potency measurements only examine the functional response. More important, potency determinations neglect a partial agonist nature of ligands, whereas efficacy calculations quantify this property. Thus, one possible explanation for the variability in efficacy of cannabinoids in the brain can be partial agonist activity or the existence of multiple subtypes of the CB1 receptor.

3.3.2. *Antagonists*

Cannabinoid research was rapidly accelerated when the first selective and potent antagonists

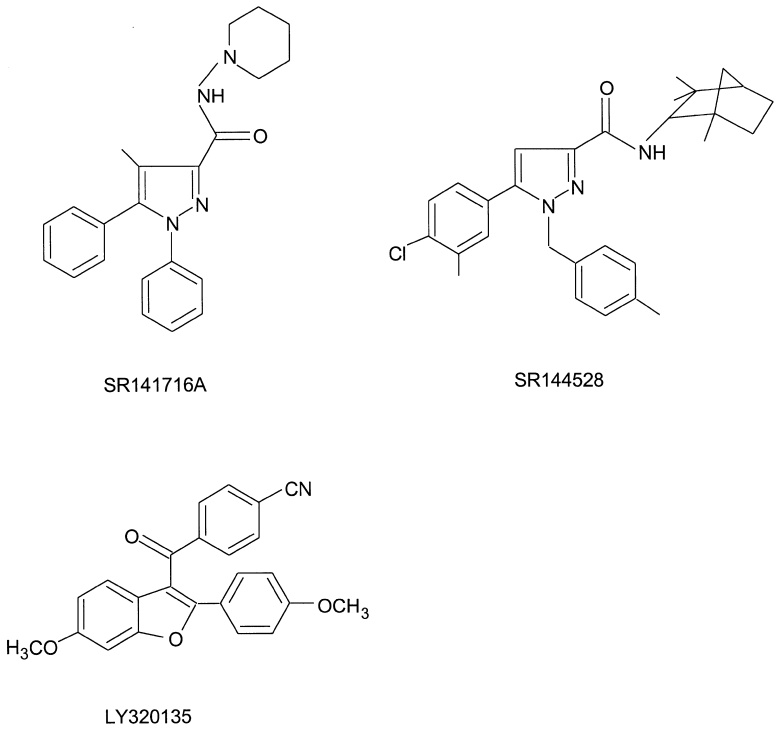

Fig. 5. Chemical structures of the cannabinoid CB1 receptor antagonists SR141716A and LY320135 as well as of the CB2 receptor antagonist, SR144528.

became available. Rinaldi-Carmona *et al.* (1994) discovered SR141716A, the most potent, well-characterized and orally active antagonist for the CB1 receptor. As shown in Fig. 5, it is chemically based on a pyrazole structure. SR141716A displays nanomolar affinity for the CB1 receptor, but micromolar affinity for CB2 in ligand binding assays (Rinaldi-Carmona *et al.*, 1994). The authors have shown that SR141716A is able to antagonize the responses to CP55 940 and WIN55 212-2 in the mous vas deferens and rat brain adenylate cyclase. In behavioral tests, it was shown to antagonize the cannabinoid-induced hypothermia, ring immobility and antinociception when applied orally. However, in some studies, SR1417116A has been reported to exert effects opposite of those produced by cannabinoid agonists and it has been suggested that it is an inverse agonist at the human CB1 and CB2 receptor expressed in Chinese hamster ovary cells (Landsman *et al.*, 1997; MacLennan *et al.*, 1998). At each subtype the basal incorporation of [^{35}S]GTPγS was reduced by SR141716. An inverse agonist, which has a preferential affinity for the inactive conformation of the receptor, will actually produce an effect opposite of an agonist, whereas a competitive antagonist has the same affinity for the active and inactive conformation and therefore does not alter the activation equilibrium.

More recently, another CB1 receptor antagonist has been described, LY320135, a substituted benzofuran (Felder *et al.*, 1998). This has a 70-fold higher affinity for the CB1 than for the CB2 receptor stably expressed in Chinese hamster ovary cells or rat spleen membranes. This compound was evaluated as a functional antagonist by its ability to reverse the inhibition by anandamide of forskolin-stimulated cAMP accumulation in the transfected cells. However, LY320135 not only reversed the anandamide-induced inhibition of cAMP production but also caused an increase in cAMP accumulation above the levels observed with forskolin alone in a similar manner to SR141716 (Felder *et al.*, 1998). LY320135 was also effective in antagonizing the effects of WIN55 212-2 on N-type calcium channels and on the inward rectifying potassium channel.

The first selective and orally active antagonist for the CB2 receptor is SR144528 (Rinaldi-Carmona *et al.*, 1998). It displays subnanomolar affinity ($K_i = 0.6$ nM) for both the rat spleen and cloned human CB2 receptors and has 700-fold lower affinity ($K_i = 400$ nM) for both the rat spleen and cloned human CB1 receptors. SR144528 antagonizes both the CP55 940-induced inhibition of adenylate cyclase and the stimulation of MAP kinase in CB2 receptor expressing Chinese hamster ovary cells. However, it failed to exert this antagonism in cells transfected with the CB1 receptor.

4. ENDOGENOUS CANNABINOIDS

The identification, pharmacological characterization, and localization of specific membrane receptors mediating the central and peripheral effects of the active principle of marijuana, Δ^9-THC, and other cannabinoids raised the question about the existence of endogenous cannabinoid agonists to which the receptors must respond, similar to the existence of endogenous ligands for the opiate recep-

tors. The only endogenous substances isolated and characterized so far that are capable of mimicking the pharmacological actions of Δ^9-THC are amides and esters of eicosanoid-like fatty-acids. To date, two eicosanoid derivatives have been isolated from both the nervous and peripheral tissues, anandamide (Devane *et al*., 1992) and 2-arachidonylglycerol (Mechoulam *et al*., 1995; Stella *et al*., 1997). Both anandamide (*N*-arachidonoylethanolamine) and 2-arachidonoylglycerol, act as true 'endogenous cannabinoids' by binding and functionally activating one or both cannabinoid receptor subtypes present on nervous and peripheral cell membranes. As shown in Fig. 4, the chemical structure of both compounds differ markedly from that of other cannabinoid receptor agonists.

4.1. Anandamide

Devane *et al*. (1992) isolated the first endogenous substance from porcine brain extracts which displayed properties expected of an endogenous cannabinoid, that is, it competed for cannabinoid receptor binding and suppressed the electrically induced twitch response of the mouse vas deferens, a characteristic effect of cannabinoid receptor agonists. By mass spectrometry, the structure of this compound was established to be *N*-arachidonylethanolamine. The name anandamide consists of the Sanskrit word *ananda*, which means 'bringer of inner bliss and tranquillity', and *amide*, the designation for the chemical bonding that distinguishes this endogenous cannabinoid from the exogenous agonists. To date, only anandamide, but none of the related fatty-acid ethanolamides have been quantified in human (Felder *et al*., 1996) and animal tissues (Devane *et al*., 1992; Schmid *et al*., 1995).

4.1.1. *Localization and Biochemistry of Anandamide*

Anandamide was isolated and quantified by liquid chromatography and mass spectrometry in various tissues of postmortem human and rat and shown to be widely distributed in the brain and periphery (Felder *et al*., 1993, 1996). Within the brain, the highest levels of anandamide were found in areas with high density of cannabinoid receptors, such as hippocampus, striatum, cerebellum and cortex. The anandamide levels in the brain are equivalent to those of other neurotransmitters such as dopamine and serotonin, but at least 10-fold lower than those reported for GABA and glutamate. The authors detected anandamide also in human and rat spleen which expresses high levels of the CB2 cannabinoid receptor, suggesting that it is an agonist at both the CB1 and the CB2 receptor. Small amounts of anandamide were also found in human heart and thalamus as well as in rat skin, whereas only trace quantities were detected in human serum, plasma and cerebrospinal fluid. The low levels found in serum, plasma, and cerebrospinal fluid suggest that it is metabolized in tissues where it is synthesized, and that its action is probably not hormonal in nature. Anandamide displays pharmacological and biochemical properties of a cannabinoid agonist for both the CB1 and CB2 receptors (Felder *et al*., 1995). Measurement of anandamide at significant levels in heart and thalamus, which are known to express few if any cannabinoid receptors may suggest that other receptors or receptor subtypes could exist for anandamide (Felder *et al*., 1996).

There is clear evidence that anandamide is a ligand of the cannabinoid receptor, since it has been shown to compete for binding to the brain CB1 receptor using radiolabeled ligands. In membrane preparations from brain and in transfected cells, it has been shown that anandamide binds to both CB1 and CB2 receptors (Devane *et al*., 1992; Munro *et al*., 1993; Felder *et al*., 1993; Slipetz *et al*., 1995). However, it has been demonstrated that the affinity of anandamide for the CB2 receptor is ca four-fold less than that for CB1 receptors (Felder *et al*., 1995; Slipetz *et al*., 1995). The K_i value reported for anandamide binding to the CB1 receptor is ca 500 nM and for binding to the CB2 receptor ca 1760 nM (Felder *et al*., 1993). Moreover, various biochemical assays provided substantial evidence that binding of anandamide to the cannabinoid receptor leads to cannabimimetic effects indicating that this eicosanoid acts as an agonist. Thus, the binding of anandamide to cannabinoid receptors causes an inhibition of adenylate cyclase. In both neuroblastoma cells and Chinese hamster ovary cells transfected with human CB1 receptor mRNA, anandamide has been demonstrated to inhibit forskolin-stimulated adenylate cyclase activity with IC_{50} values ranging from 160 to 322 nM (Felder *et al*., 1993, 1995; Vogel *et al*., 1993). In contrast anandamide failed to affect adenylate cyclase activity in non-transfected cells and in cells expressing the muscarinic M5 receptor (Felder *et al*., 1993). However, in several studies, the maximal inhibition of adenylate cyclase by anandamide was lower than the inhibition of the enzyme evoked by the synthetic cannabinoid agonists, WIN55 212-2 and CP55 940 (Vogel *et al*., 1993) indicating a lower efficacy of anandamide. Furthermore, as expected for a CB1 receptor agonist, anandamide is capable of inhibiting N-type calcium channels in both cells that naturally express cannabinoid receptors, such as N18 neuroblastoma cells (Felder *et al*., 1993; Mackie *et al*., 1993) and in AtT20 cells expressing the human CB1 receptor (Felder *et al*., 1995). However, these studies have demonstrated that anandamide is less efficacious than Δ^9-THC and WIN55 212-2 in inhibiting the N-type calcium current. In electrophysiological experiments, Mackie *et al*. (1993) have observed that the maximal inhibitory effect of anandamide on N-type calcium currents in N18 neuroblastoma cells (33%) is significantly less than that of WIN55 212-2 (54%). In experiments with anandamide in combination with WIN55 212-2, the authors have also found that the application of WIN55 212-2 always caused a further inhibition of the N-type calcium inward current in cells exposed to a maximally effective concentration of anandamide. Furthermore, the application of anandamide always caused a partial recovery of the N-type calcium current in cells exposed to a maximally effective concentration of WIN55 212-2. Thus, anandamide appears to have a lower efficacy than WIN55 212-2 in this model system. These findings imply that,

despite the inhibition of N-type calcium channels by anandamide, this eicosanoid displays the properties of a partial cannabinoid CB1 receptor agonist (Mackie *et al*., 1993). A full agonist, which has a higher affinity to the active state of a receptor than to its inactive state, will drive the equilibrium to the active state, thereby activating the receptor. The partial agonist, which is often a different but structural similar compound, binds to the same site on the receptor. But it has only a slightly greater affinity to the active conformation than to the inactive one. The consequence is a lower magnitude of effect, even at saturating concentrations, of the partial agonist.

Considering the efficacy of anandamide at the CB2 receptor, controversial results have been reported. In CB2 receptor-expressing Chinese hamster ovary cells, Felder *et al*. (1995) have observed inhibitory effects of anandamide on forskolin-stimulated cAMP accumulation. In contrast, Slipetz *et al*. (1995) failed to demonstrate an inhibition of adenylate cyclase in CB2 receptor expressing Chinese hamster ovary cells.

Anandamide is likely to produce also effects which are not mediated by cannabinoid receptors. It has been reported that anandamide is an inhibitor of gap-junction conductance and dye permeability in striatal astrocytes (Venance *et al*., 1995). Gap junctions between striatal astrocytes are well-known to conduct calcium ions between adjacent astrocytes. The calcium waves through the astrocyte network are thought to be part of a long-range signaling loop between neurons and astrocytes. Under normal conditions these calcium waves in the astrocytes are evoked by glutamate released from neurons and may, in turn, activate neurons distant from their initiation sites in astrocytes. The effect of anandamide on the function of these gap junctions has been measured using double whole-cell patch clamp recording and intracellular dye diffusion. The authors showed that anandamide blocks the propagation of astrocyte calcium waves generated by either mechanical stimulation or local glutamate application. Inhibition of these astrocytic calcium waves is sensitive to pertussis toxin and to protein-alkylating agents. Interestingly, this effect is neither mimicked by the cannabinoid receptor agonists WIN55 212-2 and CP55 940 nor prevented by the CB1 receptor antagonist SR141716A, leading to the conclusion that the effect is not mediated by the brain CB1 receptor but possibly to a non-cannabinoid, $G_{i/o}$ protein-coupled receptor. The authors concluded that, by regulating gap-junction permeability, anandamide may control intercellular communication in astrocytes and therefore neuron–glial interactions.

Anandamide has several effects which results from its conversion to arachidonic acid (see Section 4.1.2). For instance, the conversion of anandamide to arachidonic acid may be responsible for the reported inhibition of L-type calcium channels in rat brain (Shimasue *et al*., 1996), an effect that is mimicked by arachidonic acid.

The existence of specific cannabinoid receptors and the existence of an endogenous ligand which has similar brain levels like dopamine and serotonin has raised the question of whether the cannabinoid receptor system subserve the function of a neuromodulatory system with anandamide as neurotransmitter. The functional requirements, however, for a classical neurotransmitter include the synthesis, storage and release into the synaptic cleft of the supposed transmitter substance from nerve terminals. However, unlike classical neurotransmitters which are stored in synaptic vesicles anandamide seems to be much too hydrophobic to be stored within synaptic vesicles and it is expected to pass easily through plasma membranes (Axelrod and Felder, 1998). Due to its physical properties, it may act at both cell membrane and intracellular compartments. Therefore, anandamide deviates from the classical definition of neurotransmitters. On the other hand, anandamide has been reported to be released from cultured striatal and cortical neurons but not from astroglia cultures in a calcium ion-dependent manner when these neurons were stimulated with membrane-depolarizing agents or glutamatergic receptor stimulation (Di Marzo *et al*., 1994) supporting the premise that anandamide could have some properties characterizing a neurotransmitter or neuromodulator.

4.1.2. *Biosynthesis and Metabolism of Anandamide*

One of the qualifications of a neurotransmitter system is the existence of a path for synthesis and degradation of the ligand. Indeed, soon after the discovery of anandamide, enzymatic activities responsible for its biosynthesis and degradation were described (Deutsch and Chin, 1993; Devane and Axelrod, 1994; Di Marzo *et al*., 1994; Kruszka and Gross, 1994; Ueda *et al*., 1995). A catalytic activity for the biosynthesis of anandamide from ethanolamine and arachidonic acid was readily apparent in incubations of rat brain homogenates.

Two hypotheses have tried to explain the biosynthesis of anandamide. Previously, it has been proposed that anandamide is synthesized via condensation of arachidonic acid and ethanolamine in a reaction catalyzed by a putative anandamide synthase (Devane and Axelrod, 1994; Kruszka and Gross, 1994). However, kinetic measurements revealed that the concentrations of both free arachidonic acid and ethanolamine normally present in tissue are not as high as necessary for this reaction to proceed (Devane and Axelrod, 1994; Kruszka and Gross, 1994). A plausible biochemical pathway which is believed to be involved in the anandamide biosynthesis has been proposed to occur in the brain (Sugiura *et al*., 1996; Cadas *et al*., 1997). This hypothesis suggests that anandamide may be stored in the lipid layer of the plasma membrane, esterfied as *N*-arachidonylphosphatidylethanolamine. As shown in Fig. 6, the formation of *N*-arachidonylphosphatidylethanolamine is believed to occur through a series of enzymatic rearrangements of membrane lipid components. According to the biochemical pathway proposed by Cadas *et al*. (1997), an *N*-acyltransferase is likely to be activated by rises in the intracellular calcium concentration produced by neuronal activity. *N*-Acyltransferase activity is enriched in brain and testis. this enzyme catalyzes

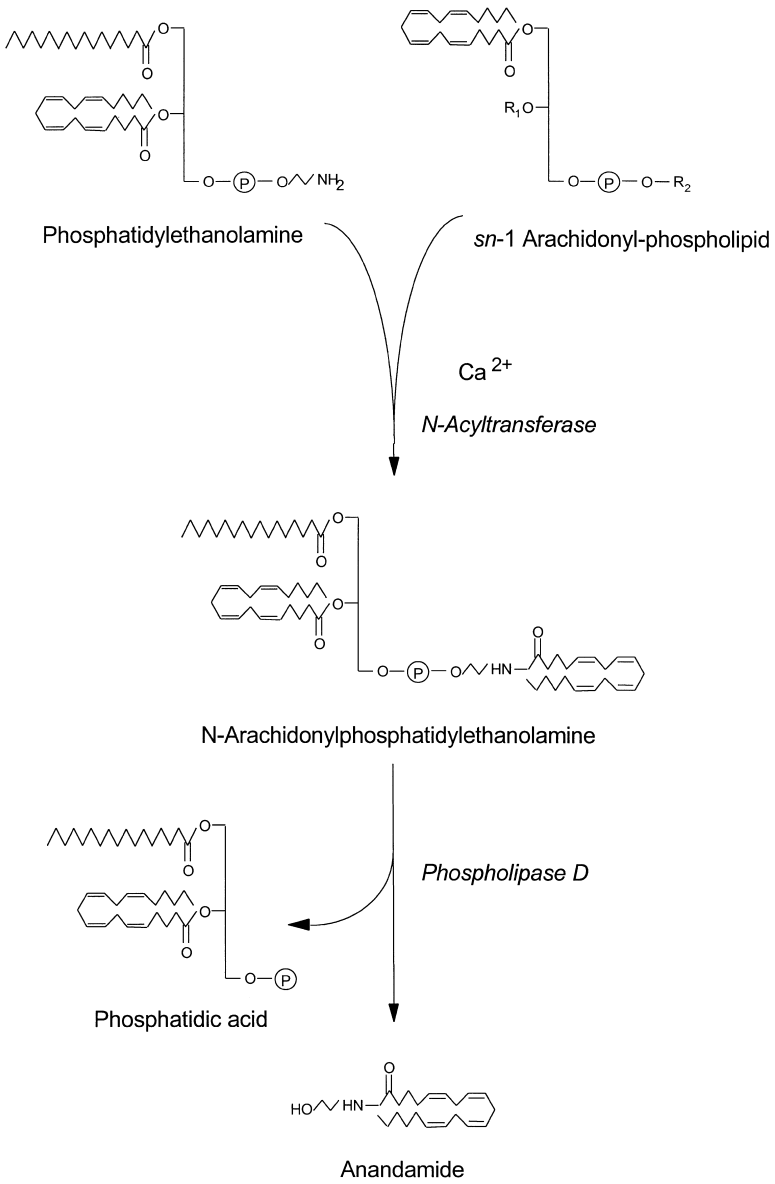

Fig. 6. Proposed pathway for the formation of anandamide through a series of enzymatic rearrangements of membrane lipid components in brain neurons. According to this model, an increase in the intracellular calcium concentration elicited by neuronal activity stimulates *N*-acyltransferase, which catalyzes the transfer of arachidonic acid from a donor lipid (*sn*-1 arachidonyl-phospholipid) to phosphatidylethanolamine by forming the amide bond and, thereby the putative anandamide precursor molecule, *N*-arachidonylphosphatidyethanolamine. Phospholipase D cleaves the phosphodiester bond of *N*-arachidonylphosphatidyethanolamine and anandamide is released.

the transfer from arachidonic acid from the first position of a donor phospholipid (*sn*-1 arachidonyl-phospholipid) to the third position of phophatidylethanolamine where an amide bond is formed. This step generates the putative anandamide storage molecule, *N*-arachidonylphosphatidylethanolamine. Cadas *et al*. (1997) have also shown that *sn*-1arachidonyl-phospholipids are present in brain, where they constitute ca 0.5% of total phospholipids. Calcium ions may also stimulate phophodiesterase activity (phospholipase D), which is assumed to cleave the distal phosphodiester bond, thereby releasing anandamide. Since anandamide is a highly lipophilic compound, it is unlikely to be stored in synaptic vesicles. Considering the pathway of its biosynthesis, it seems possible that anandamide resides in the membrane, stored in form of the putative precursor, *N*-arachidonylphosphatidylethanolamine, to be released following activation of phospholipase D.

However, two questions raise to this pathway proposed by Sugiura *et al*. (1996) and Cadas *et al*.

(1997). First, the tissue distribution of *N*-acyltransferase only partially parallels that of CB1 receptors. As described in Section 3.1.1., these receptors are densely expressed in cortex, hippocampus, cerebellum and striatum, where *N*-acyltransferase activity is also high. But they are only sparsely expressed in the brain stem, where the authors have found the highest *N*-acyltransferase activity. A possible explanation would be that anandamide has additional effects which are not mediated by the CB1 and CB2 receptors (Venance *et al*., 1995). Second, in brain, the levels of *N*-arachidonylphosphatidylethanolamine are only twofold higher than the levels of anandamide, suggesting that either this lipid does not represent an anandamide storage molecule or that anandamide formation needs additional stimulation by calcium.

As a putative neuromodulator, anandamide, which is released into the synaptic cleft, is expected to be rapidly inactivated. In general, two possibilities are known to remove a transmitter from the synaptic cleft to ensure rapid signal inactivation, that is, either re-uptake or enzymatic degradation. Deutsch and Chin (1993) showed that anandamide is rapidly taken up by neuroblastoma cells and hydrolyzed to arachidonic acid and ethanolamine by an amidase. Degradation also was observed in brain, heart, kidney and lung tissues. The authors discovered phenylmethylsulfonyl fluoride to be a potent inhibitior of the enzymatic breakdown of anandamide. More recently, it has been reported that anandamide is hydrolyzed by a widely distributed and non-selective fatty-acid amidohydrolase which also degrades a sleep-inducing oleamide first isolated from sleep-deprived cats (Cravatt *et al*., 1996). However, fatty-acid amidohydrolase activity has been only found in the intracellular compartments of neurons but not in the plasma membrane. This means that the enzymatic breakdown of released anandamide must be preceded by an uptake into the neuron. Although anandamide is a highly hydrophobic and thus could gain access to the fatty-acid amidohydrolase by passive diffusion, the transfer rate is expected to be low because of the molecular size of this molecule. Beltramo *et al*. (1997) have demonstrated the existence of a rapid, saturable transmembrane carrier, which is selectively blocked by *N*-(4-hydroxyphenyl)arachidonylamide (AM404). AM404 is a structural analogue of anandamide and acted as a competitive inhibitor, suggesting that it may serve as a transport substrate or pseudosubstrate. It is both efficacious and relatively potent. The IC_{50} for the accumulation of [^{3}H]anandamide is 1 μM in neurons and 5 μM in astrocytes (Beltramo *et al*., 1997). The authors have shown that although AM404 does not activate cannabinoid receptors or inhibit anandamide hydrolysis, it is capable of enhancing the cannabinoid receptor-mediated effects of anandamide *in vitro* and *in vivo*. This finding indicate that a high affinity transport system present in neurons and astrocytes has a role in the breakdown of anandamide by removing this lipid mediator from the extracellular space and delivering it to intracellular metabolizing enzymes such as fatty-acid amidohydrolase (Beltramo *et al*., 1997).

In conclusion, the ability of brain tissue to enzymatically synthesize and metabolize anandamide as well as the existence of a carrier-mediated transport mechanism essential for the termination of the biological effects of anandamide and of specific receptors for this eicosanoid suggest the presence of anandamide-containing (anandaergic) neurons. Therefore, anandamide may represent a member of a new family of fatty-acid-derived neuromodulators.

4.1.3. *Biological Activity of Anandamide*

Since the discovery and isolation of anandamide its biological activity was compared to that of other cannabinoids, in different model systems. *In vivo*, anandamide produces a pharmacological profile very similar to that of the classical cannabinoid receptor agonists, including antinociception, hypothermia, hypomobility, and catalepsy in mice after i.v., i.t. and intraperitoneal (i.p.) administration (Crawley *et al*., 1993; Fride and Mechoulam, 1993; Smith *et al*., 1994a; Calignano *et al*., 1998). In general, the effects of anandamide occurred with a rapid onset, but with a rather short duration of action. The short duration of action is likely to be due to the rapid uptake into neurons and astrocytes and subsequent enzymatic degradation as reported above. Maximal effects on motor activity (ca 85% inhibition) and prominent antinociceptive effects ($>80\%$ maximal possible effect) were measured immediately after i.v. and i.t. administration (Smith *et al*., 1994a). A pharmacological comparison between anandamide and Δ^9-THC revealed that anandamide not only has a shorter duration of action than Δ^9-THC but also was 4–20-fold less potent in producing these pharmacological effects (Smith *et al*., 1994a). Despite the relatively short duration of the anandamide-induced effects following a single injection, the development of tolerance to the administration of anandamide has been established in mice. When mice have received an i.p. injection of a high dose of anandamide (40 mg kg^{-1}) four times per day for 3 days, tolerance developed to the anandamide-induced antinociception. Anandamide-tolerant mice were also cross-tolerant to Δ^9-THC-induced analgesia (Welch, 1997). Anandamide has also been reported to mimick several of the peripheral effects of Δ^9-THC. In anaesthetized rats, anandamide presented potent cardiovascular effects which included a short pressor effect and a prolonged depressor response (Varga *et al*., 1996). Only the depressor but not the pressor response was inhibited by the CB1 receptor antagonist SR141716A. This suggested the existence of distinct mechanisms of anandamide action which include a receptor-mediated (the depressor response) and a CB1-receptor-independent (the pressor component) modes of action.

While the above evidences provide ample support for anandamide displaying cannabimimetic effects, there are some differences between Δ^9-THC and anandamide. Smith *et al*. (1994a) have reported that the antinociception produced by anandamide, in contrast to that evoked by Δ^9-THC, can not be blocked by pretreatment with the κ-opioid receptor antagonist nor-binaltorphimine. Furthermore, these authors have reported that, also unlike Δ^9-THC and

other classical cannabinoids, anandamide fails to display an antinociceptive action when administered i.c.v. This finding suggests the existence of distinct differences between anandamide and Δ^9-THC with regard to their antinociceptive properties. Interestingly, several recent studies reported that the anandamide-induced antinociception can not be prevented by SR141716A (Adams *et al*., 1998; Smith *et al*., 1998b; Vivian *et al*., 1998). Thus, the anandamide-induced antinociceptive effect does not result from CB1 receptor stimulation.

Considering the anandamide's contribution to the control of pain transmission, surprising new findings have just been reported by Calignano *et al*. (1998). These authors showed by use of the formalin test that anandamide attenuates the pain behavior produced by chemical damage to cutaneous tissue by interacting with CB1-like receptors located outside the CNS. The analgesic effects of anandamide were prevented by the CB1 receptor antagonist SR141716A, but not by the CB2 antagonist SR144528 or by the opioid antagonist naloxone. Anandamide was more potent in preventing formalin-evoked pain behavior when injected locally into the paw rather than i.v. In the same study, the authors have observed that radiolabeled anandamide remained associated with the injected paw and did not diffuse to brain or spinal cord, indicating that anandamide inhibits nociception after formalin injection by activating CB1 receptors located on peripheral endings of sensory neurons. Furthermore, palmitylethanolamide, which is released together with anandamide from the putative precursor, *N*-arachidonylphosphatidylethanolamine, also inhibited formalin-induced pain behavior. However, this effect was inhibited by SR144528, but not by SR141716A or naloxone, indicating the involvement of CB2 receptors. After coadministration of both anandamide and palmitylethanolamide the antinociceptive potency of each compound was increased 100-fold. These results indicate that the simultaneous activation of peripheral CB1 and CB2 receptors results in a synergistic inhibition of peripheral pain transmission. This is in line with the finding that both SR141716A and SR144528 have hyperalgesic effects in the same model (Calignano *et al*., 1998). The authors proposed that this hyperalgesia is due to the removing of an endogenous cannabinoid tone, since gas-chromatography/mass spectrometry analyses revealed that the levels of anandamide (ca 50 pmol g^{-1}) and palmitylethanolamide (ca 700 pmol g^{-1}) in the skin are five- to ten-fold higher than in the brain, and high enough to cause a tonic activation of local cannabinoid receptors.

Taken together, the endogenous eicosanoid, anandamide, is synthesized in neurons, released upon depolarization and rapidly inactivated in neurons and glia cells. Following release it is able of activating CB1 and, with less affinity, CB2 receptors. Although ample evidences exist supporting the cannabimimetic function of anandamide, future studies are expected to give further insight into the physiological and pathophysiological role of the anandamide–cannabinoid receptor system. Some subtle differences in the action of anandamide and classical cannabinoids exist, suggesting that, in some cases, anandamide may act as a partial agonist, and this, in turn, implies the existence of other endocannabinoids. Of particular interest is the possibility that anandamide and other structurally related fatty-acid derivatives represent a new class of lipid neurotransmitters.

4.2. 2-Arachidonylglycerol

With the finding of the first endocannabinoid, the hypothesis of the existence of an endogenous cannabinoid system was pushed forward for the first time after the cloning and molecular characterization of the brain CB1 receptor in 1990 by Matsuda *et al*. (1990). Indeed, a second phospholipid-derived messenger, the monoacylglycerol 2-arachidonylglycerol, has more recently been identified and reported to display properties of an endogenous cannabinoid receptor agonist (Stella *et al*., 1997). Considering its chemical structure, this new putative endocannabinoid is an arachidonyl ester rather than an amide (Fig. 4). Already previously, 2-arachidonylglycerol has been isolated from canine intestine (Mechoulam *et al*., 1995). With K_i values of 2-arachidonylglycerol for the CB1 receptors are reported to be in the range of 0.7–1.2 μM (Mechoulam *et al*., 1995). Probably due to the recent discovery of cannabimimetic properties of 2-arachidonylglycerol, still very few is known about the physiological role of this monoacylglycerol. In the presence of forskolin, 2-arachidonylglycerol inhibited adenylate cyclase in isolated mouse spleen cells, at the potency level of Δ^9-THC. In cultured cortical neurons, 2-arachidonylglycerol has been shown to inhibit the forskolin-stimulated cAMP accumulation with an IC_{50} of 0.8 μM (Stella *et al*., 1997). This effect displayed sensitivity to pertussis toxin and SR141716A, clearly indicating that 2-arachidonylglycerol is capable of activating the CB1 receptor and, therefore, representing a second endogenous modulator of this receptor. Moreover, Stella *et al*. (1997) reported that 2-arachidonylglycerol prevents the induction of long-term potentiation at the CA3–CA1 synapse, a model for learning and memory, in hippocampal slices without affecting basal synaptic transmission. This effect was prevented by SR1411716A. Upon i.v. administration to mice, 2-arachidonylglycerol caused the typical tetrad of effects produced by Δ^9-THC, that is, antinociception, immobility, reduction of spontaneous activity, and lowering of the rectal temperature. Taken together, these findings suggest a neuromodulatory role of for the monoglyceride. 2-Arachidonylglycerol also shares the ability of Δ^9-THC to inhibit electrically evoked contractions of mouse isolated vasa deferentia; however, it is less potent than Δ^9-THC (Mechoulam *et al*., 1995).

Although 2-arachidonylglycerol was initially isolated from peripheral tissue, it has been recently confirmed by use of reverse-phase high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry that 2-arachidonoylglycerol is one of the most abundant molecular species of monoacylglycerols in the brain (Stella *et al*., 1997; Kondo *et al*., 1998). Substantial amounts of 2-arachidonoylglycerol were also found in the liver,

spleen, lung and kidney, but the levels were considerably lower than that in the brain (Kondo *et al*., 1998). Stella *et al*. (1997) have found 2-arachidonoylglycerol to be present in brain in amounts 170 times greater than anandamide. Interestingly, these authors discovered that neuronal activity evoked by high-frequency stimulation of hippocampal slices produced a four-fold increase in the levels of 2-arachidonylglycerol compared with the levels in absence of electrical stimulation. However, in contrast to 2-arachidonylglycerol, the anandamide levels are unaffected by the high frequency stimulation (Stella *et al*., 1997). The enhanced production of 2-arachidonylglycerol was abolished by both the sodium channel blocker tetrodotoxin and by omission of calcium ions indicating that the formation or release of this lipid messenger requires both neuronal depolarization and external calcium. More recently, the requirement of calcium for the biosynthesis and release of 2-arachidonylglycerol has been observed also in mouse neuroblastoma cells (Bisogno *et al*., 1998) and in a brain homogenate (Kondo *et al*., 1998), supporting the previous findings of Stella *et al*. (1997) obtained at cultured rat cortical neurons and electrically stimulated hippocampal slices that calcium ions play a key role in regulation of the generation of 2-arachidonoylglycerol in brain.

Three main biochemical pathways seem to exist for the formation of 2-arachidonylglycerol, which seems to be tissue-selective. In neurons, biosynthesis of 2-arachidonylglycerol may occur from the breakdown of phospholipids in membranes through the sequential action of a phophoinositide-specific phospholipase C and *sn*-1-diacylglycerol lipase (Stella *et al*., 1997; Bisogno *et al*., 1998). In an initial step, phospholipase C leads to the parallel release of diacylglycerol and a diarachidonylphospholipid. The latter compound is subsequently hydrolyzed by *sn*-1-diacylglycerol lipase to yield 2-arachidonylglycerol. It has been proposed that there is a possible connection in the biosynthesis pathways of 2-arachidonylglycerol and anandamide (Bisogno *et al*., 1998; Di Marzo *et al*., 1998), since a diarachidonylphospholipid may serve as arachidonic acid donor for the *N*-acyltransferase-mediated formation of the anandamide precursor, *N*-arachidonylphosphatidylethanolamine. The by-product of this calcium-dependent trans-acylation is a *sn* 1-lyso-2-arachidonylphospholipid, which can be converted into 2-arachidonylglycerol by phospholipase C.

While it is well-known that the breakdown of anandamide is catalyzed by fatty-acid amidohydrolase (Cravatt *et al*., 1996), the enzyme responsible for the hydrolysis of 2-arachidonylglycerol to gycerol and arachidonic acid has not yet been discovered. Di Marzo *et al*. (1998) have recently reported that 2-arachidonylglycerol is rapidly inactivated and recycled by mouse neuroblastoma cells and intact rat basophilic leukaemia cells through a process which involves diffusion, hydrolysis and reacylation. It seems, that in contrast to the hydrolysis of anandamide by fatty-acid amidohydrolase, several esterases, including fatty-acid amidohydrolase, are capable of recognizing 2-arachidonylglycerol as a substrate (Di Marzo *et al*., 1998). This would again raise the possibility of cross-regulatory mechanisms between these two endogenous cannabinoids.

In conclusion, a neuromodulatory role for 2-arachidonylglycerol is strongly supported also by the finding of molecular mechanisms for its calcium-dependent biosynthesis following neuronal stimulation either and for its enzymatic degradation.

5. MODULATION OF NEURONAL ACTIVITY IN DISTINCT BRAIN REGION

This section focuses on the effects induced by cannabinoids via modulation of three main transmitters, glutamate, dopamine, and GABA, in such brain regions known to possess a high density of CB1 receptors and to be involved in some of the characteristic effects of marijuana.

5.1. Basal Ganglia

One of the most striking behavioral effects of plant and synthetic cannabinoids as well as of endogenous cannabinoids is the depression of locomotor activity. Brain regions involved in the control of movement, such as the cerebellum and the basal ganglia have been demonstrated to possess the highest density of CB1 receptors within the brain (Herkenham *et al*., 1990, 1991a; Mailleux and Vanderhaeghen, 1992). Figure 7 illustrates the organization of the basal ganglia and the intrinsic connections. The basal ganglia consists of five nuclei, that is, nucleus caudatus, putamen, globus pallidus, nucleus subthalamicus and substantia nigra. The nucleus caudatus and putamen form together the striatum. The striatum receives glutamatergic projections from motoric and sensoric cortical areas and is the most important input nucleus of the basal ganglia. The most important output nuclei are the globus pallidus and the substantia nigra pars reticulata. Within the basal ganglia, cannabinoid receptors occur both pre- and postsynaptically. In the rat as well as in the human brain, the output nuclei of the basal ganglia, that is, the globus pallidus and substantia nigra pars reticulata, exhibit extremely high levels of cannabinoid receptors (Herkenham *et al*., 1990, 1991a; Mailleux and Vanderhaeghen, 1992). Cannabinoid receptors in these nuclei are assumed to be predominantly localized on presynaptic terminals of the GABAergic striatonigral and striatopallidal terminals. This assumption is supported by two major experimental findings: first, neither in the substantia nigra nor in the globus pallidus significant levels of mRNA for the cannabinoid receptor has been detected (Mailleux and Vanderhaeghen, 1992; Westlake *et al*., 1994), suggesting that the receptors occur presynaptically on afferent striatal terminals. Second, destruction of striatal neurons by ibotenate has been shown to cause a loss of cannabinoid receptor binding in the globus pallidus and substantia nigra, the target zones of striatal neuronal projections. This finding indicate that CB1 receptors are located presynaptically on the degenerating terminal of the striatal projection neuron. Cannabinoid receptors have been shown to coexist with both dopamine D_1 and D_2

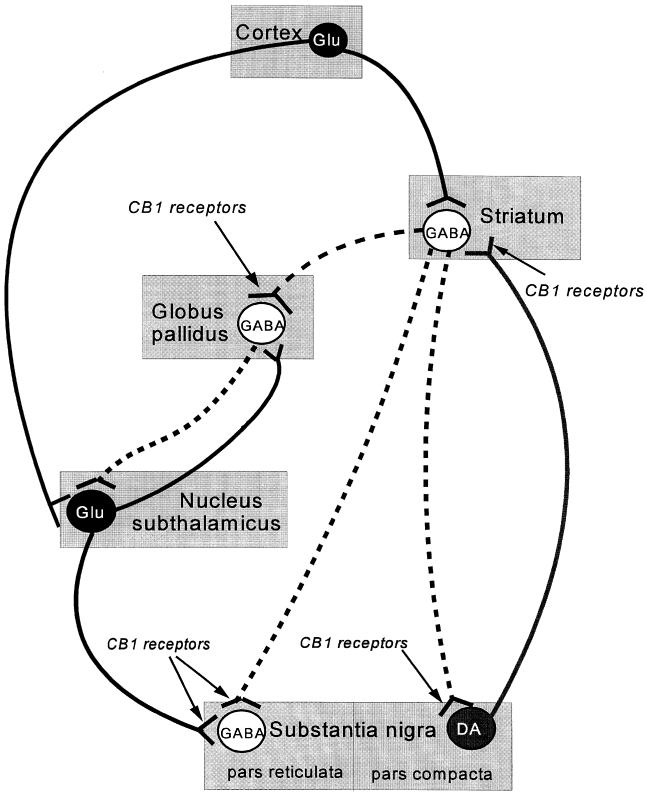

Fig. 7. Neurotransmission in the basal ganglia. Locomotor activity depends on the balance of inhibitory and excitatory systems. This balance is modulated by endogenous cannabinoids acting at CB1 receptors which have high densities within the basal ganglia. Cannabinoids are thought to decrease locomotor activity by an inhibition of GABA uptake at striatopallidal and striatonigral terminals, by inhibition of dopamine (DA) uptake in the striatum and, finally, by a decrease in glutamate (Glu) release from the terminals of the nucleus subthalamicus, thereby inhibiting the firing of the substantia nigra.

receptors on striatonigral and striatopallidal terminals (Herkenham *et al.*, 1991a; Mansour *et al.*, 1992) and apparently produce opposite effects on second messengers and neurotransmitter release, that is, CB1 receptor activation is able to inhibit a D_1-mediated increase in cAMP accumulation (Bidaut-Russell and Howlett, 1991) and also able to inhibit the D_2-mediated inhibition of cAMP accumulation (Glass and Felder, 1996). The behavioral implications of manipulation of dopaminergic transmission by activation of cannabinoid receptors are not yet very well characterized. It has been reported that unilateral intrastriatal injection of classical cannabinoids as well as of anandamide produces contralateral turning behavior (Souilhac *et al.*, 1995). This effect was antagonized by SR141716A, clearly indicating that it is CB1 receptor-mediated. On the other hand, it was suppressed by prior 6-hydroxydopamine lesions of the striatum, indicating that it is dopamine-mediated (Souilhac *et al.*, 1995). Since unilateral increase in dopamine transmission in the striatum is well known to induce contralateral turning behavior, it may be assumed that cannabinoids increase the dopamine level in the striatum by an inhibition of its reuptake. An enhanced release of dopamine can be excluded considering the inhibitory effects of cannabinoids on *N*-type calcium channels (Mackie and Hille, 1992; Mackie *et al.*, 1993), which are responsible for neurotransmitter release (see Section 3.1.2.2).

Furthermore, unilateral intranigral injections of cannabinoids also have been reported to provoke contralateral turning behavior (Sañudo-Peña *et al.*, 1996). This effect seems to be the result of an inhibition of glutamate release from subthalamic nucleus terminals, the excitatory input to the substantia nigra. The nucleus subthalamicus receives glutamatergic projections from motoric cortical areas. Within the basal ganglia, this nucleus is connected with the globus pallidus and the substantia nigra pars reticulata, to which it sends glutamatergic projections. The nucleus subthalamicus receives GABAergic input from the pallidum (see also Fig. 7). Cannabinoids have been shown to inhibit the increased firing in the substantia nigra produced by stimulation of the subthalamic nucleus (Sañudo-Peña and Walker, 1997). Glutamate is the predominant excitatory neurotransmitter in the basal ganglia, and the subthalamic nucleus sends glutamatergic projections to the output nuclei of the basal ganglia. With regard to the findings that cannabinoids block N- and P/Q-type calcium channels, it is conceivable that cannabinoids inhibit the glutamate release from the subthalamic efferent terminals in the output regions of the basal ganglia by binding to CB1 receptors located on these terminals. This

assumption is supported by the presence of CB1 receptor mRNA in the subthalamic nucleus (Matsuda *et al.*, 1993). Inhibition of glutamate release from the subthalamic terminals in the output regions of the basal ganglia could have therapeutic implications, as in Parkinson's disease both are overactive and disturb the neurochemical balance of the basal ganglia.

Cannabinoid receptors in basal ganglia are located presynaptically on terminals arising from the striatum that use GABA as neurotransmitter. More than 85% of the CB1 receptors in the globus pallidus are located on those GABAergic terminals (Herkenham *et al.*, 1991a). It has been shown previously that Δ^9-THC injected into the globus pallidus induces catalepsy through interactions with GABAergic pathways (Pertwee and Wickens, 1991). Catalepsy is a typical effect of cannabinoids in animals and is thought to be due to an interference of cannabinoids with extrapyramidal motor systems (Gough and Olley, 1978; Moss *et al.*, 1981; Compton *et al.*, 1991; Abood and Martin, 1992; Howlett, 1985). Several studies have recently investigated the possible role of these CB1 receptors in modulating GABAergic transmission (Maneuf *et al.*, 1996; Romero *et al.*, 1996, 1998b; Szabo *et al.*, 1998). The cannabinoid-induced motor inhibition is reversed by $GABA_B$, but not by $GABA_A$ receptor antagonists (Romero *et al.*, 1996), indicating an increased GABA concentration in the synaptic cleft of the $GABA_B$ receptor synapse. It has been further shown that this increased GABA transmission is the result of decreased reuptake of GABA into the presynaptic terminal rather than increased GABA release or decreased GABA synthesis (Maneuf *et al.*, 1996; Romero *et al.*, 1998b; Szabo *et al.*, 1998). The increase of the GABA concentration in the synaptic cleft leads to a potentiation of inhibitory GABA action on neurons receiving striatal GABAergic innervation, mainly nigrostriatal dopaminergic neurons and results in marked motor inhibition. It is assumed that the mechanisms leading to catalepsy involve an increase in GABAergic transmission in basal ganglia resulting from blockade of GABA uptake by cannabinoids.

The occurrence of CB1 receptors in the basal ganglia and the effects of cannabinoids in these regions imply that endogenous cannabinoids may play an essential role in the fine tuning of motor control. Indeed, several reports have demonstrated disturbances in CB1 receptor expression and binding in neurological disorders of the extrapyramidal system. Thus, cannabinoid receptor binding is decreased in neurodegenerative diseases, such as Parkinson's disease and Huntington's disease (Glass *et al.*, 1993; Richfield and Herkenham, 1994; Sañudo-Peña *et al.*, 1998). Autoradiographic studies with postmortem brains from patients suffering of Huntington's disease revealed a marked loss of CB1 receptors at the striatal nerve terminals, presumably reflecting loss of striatonigral neurons (Glass *et al.*, 1993; Richfield and Herkenham, 1994). The degree of loss of cannabinoid binding sites was found to correlate with the grade of neuropathological severity. Interestingly, aged rats have recently been reported to show a marked decrease in cannabinoid receptor binding in most of the basal ganglia, excepting the globus pallidus which had similar levels in aged and young rats (Romero *et al.*, 1998a). Furthermore, the authors reported that aged rats also exhibited significant reductions in the CB1 receptor mRNA levels in the medial and lateral caudate putamen, the areas where the cell bodies of CB1 receptor-containing neurons projecting to the substantia nigra and globus pallidus are located. This findings indicate, that synthesis and binding levels of CB1 receptors decrease in different structures of the extrapyramidal system during aging.

In conclusion, it appears that both exogenous and endogenous cannabinoids exert powerful influence on nigral physiology by presynaptic control over both its excitatory and inhibitory inputs, thus playing a major role in the regulation of this important output structure of the basal ganglia.

5.2. The Mesolimbic Dopaminergic System

Although marijuana is so widely abused, the mechanisms of its euphoriant and addictive effects are still unknown. The rewarding (reinforcing) properties of a number of commonly abused psychoactive drugs are brought by activation of the mesolimbic dopaminergic reward system which ascends from the ventral tegmental area of the midbrain (VTA) to rostral structures (Fig. 8); of these, the nucleus accumbens is of particular importance in drug addiction (for review see Herz, 1997). The somata of the dopaminergic neurons (A10) are located in the VTA, while the nucleus accumbens represents an important terminal field of fibers arising in the VTA. As shown in Fig. 9, the mesolimbic dopaminergic system is modulated by endogenous opioids (Koob, 1992; Herz, 1997). Increase in dopamine release in the nucleus accumbens is caused by

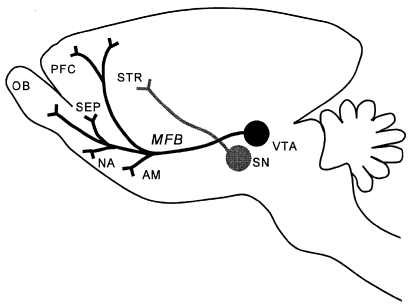

Fig. 8. Localization of dopaminergic neurons and their projections in the rat brain. The nigrostriatal fibers (*gray*) project from the substantia nigra to the striatum. The dopaminergic median forebrain bundle (*black*) mediates the reinforcing properties of the most drugs of abuse. The ventral tegmental area and the nucleus accumbens represent key structures of the dopaminergic mesolimbic reward system. The somata of the dopaminergic neurons are located in the ventral tegmental area, while the nucleus accumbens represents an important terminal field of fibers arising in the ventral tegmentum. AM, amygdala; MFB, medial forebrain bundle; NA, nucleus accumbens; OB, olfactory bulb; PFC, prefrontal cortex; SEP, septum; SN, substantia nigra; STR striatum; VTA, ventral tegmental area.

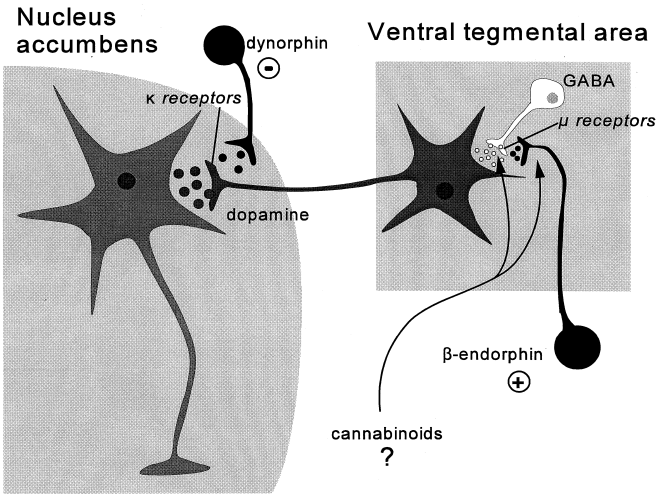

Fig. 9. Illustration indicating the regulation and modulation of the mesolimbic dopaminergic reward system by endogenous opioids and GABA. The dopaminergic neurons in the ventral tegmental area are under control of inhibitory GABAergic interneurons. The ventral tegmental area receives projections of the tonically active β endorphinergic system. Disinhibition of the GABA tonus by presynaptic μ opioid receptors results in an increased dopamine release at the terminals of the dopaminergic neuron (A10) in the nucleus accumbens. The dopamine release in the nucleus accumbens is under tonic control of presynaptic dynorphinergic terminals. Dynorphin activates κ opioid receptors which inhibit dopamine release by hyperpolarization. Cannabinoids increase the dopamine release in the nucleus accumbens by an unknown mechanism. Two possible actions are conceivable: direct interaction at the GABAergic terminals, thereby decreasing the GABA release, or interaction with the β endorphinergic system, thereby enhancing the disinhibition.

tonic activation of μ and probably δ opioid receptors in the VTA, whereas tonic activation of κ opioid receptors in the nucleus accumbens itself mediate a decrease of dopamine release. This means, there is a tonic control of dopamine release by rewarding μ and δ receptors as well as by aversive κ receptors, which have deciding influence on mood and motivation. Morphine (Koob, 1992), ethanol (Phillips and Shen, 1996; Herz, 1997) and nicotine (Schilstrom *et al*., 1998) have been demonstrated to increase the discharge rate of the mesolimbic dopaminergic neurons, thereby increasing the dopamine output in the nucleus accumbens. Activation of the opioid receptors in the VTA by, for example, morphine or heroin blocks the activity of the GABAergic interneurons, thereby producing disinhibition and increased firing of the dopaminergic neurons, and, in turn, enhanced dopamine release in the nucleus accumbens. Indeed, there is a compelling body of evidence that Δ^9-THC also increases dopamine activity in reward relevant circuits in the brain, in particular the mesolimbic pathway projecting from the VTA to the nucleus accumbens. *In vivo* studies have shown that Δ^9-THC increases extracellular concentrations of dopamine in the nucleus accumbens in a tetrodotoxin-sensitive and calcium-dependent manner (Chen *et al*., 1990). More recently, it has been shown by brain microdialysis that Δ^9-THC increases the extracellular dopamine concentration preferentially in the shell of the nucleus accumbens, but not in the core, similar to the action of heroin (Tanda *et al*., 1997). This effect was attributed to an action on CB1 receptors, because it was prevented by SR141716A. Given the extensive connections of the shell with limbic brain areas involved in emotion, the activation of dopamine transmission in the shell of the nucleus accumbens may be involved in the affective and motivational properties of Δ^9-THC. Furthermore, systemic administration of Δ^9-THC as well as synthetic cannabinoids produce an increase in spontaneous firing of dopamine neurons within the VTA, whereas the non-psychoactive compound cannabidiol and the inactive enantiomers of cannabinoid receptor agonist did not affect the firing (French, 1997; French *et al*., 1997; Gessa *et al*., 1998). The excitatory effect of psychoactive cannabinoids on mesolimbic dopamine neuronal firing is prevented by pretreatment with SR141716A (French, 1997), indicating CB1 receptor-mediated effect. However, although the increasing in firing may explain the increases in extracellular dopamine levels after Δ^9-THC administration (Chen *et al*., 1990), the exact site and the mechanisms mediating this stimulatory effect are still unknown. A direct action of Δ^9-THC seems to be ruled out, because cannabinoid receptors are not localized on dopamine neurons (Herkenham *et al*., 1991a,b). One possible mechanism of action of cannabinoids in the VTA-nucleus accumbens pathway is a cannabinoid-induced inhibition of GABAergic interneurons within the VTA (Fig. 9), leading to disinhibition of dopamine cell firing. An alternative possibility is an interaction with the endogenous opioid system, which is known to modulate the dopamine release. Interestingly, Tanda *et al*. (1997) reported that the increase in extracellular dopamine concentration in the shell of the nucleus accumbens evoked by Δ^9-THC as well as by heroin is prevented by the μ_1 opioid receptor antagonist naloxonazine. The authors suggested that Δ^9-THC and heroin exert

similar effects on mesolimbic dopamine transmission through a common μ_1 opioid receptor mechanism located in the ventral mesencephalic tegmentum. Recently, it has been shown that subchronically (5 days) administration of Δ^9-THC (10 mg kg^{-1}-day^{-1}) increases proenkephalin mRNA levels in the nucleus accumbens of rats by 25% (Manzanares *et al.*, 1998). This result reveals that chronic cannabinoid administration increases opioid gene expression in the nucleus accumbens and suggests an interaction between the cannabinoid and enkephalinergic system. It is expected, therefore, that the effects of cannabinoids on the mesolimbic dopaminergic system are complex.

In conclusion, psychoactive cannabinoids increase the activity of dopaminergic neurons in the VTA-mesolimbic pathway by a still unknown mechanism. Since these dopaminergic circuits are known to play a pivotal role in mediating the reinforcing effects of most drugs of abuse, the enhanced dopaminergic drive elicited by the cannabinoids could underlie the abuse properties of marijuana.

5.3. Hippocampus

Great interest has been generated in the effects of cannabinoids on memory and cognition. Chronic exposure to Δ^9-THC or marijuana extracts persistently alters the structure and function of the rat hippocampus, a paleocortical brain region involved in learning and memory processes in both rats and humans (Scallet, 1991). Indeed, disruptive effects of cannabinoids on memory and cognition are the most frequently described findings (see Section 2.2.1). The cognitive disruption by Δ^9-THC in this brain structure has been attributed to the high density of CB1 receptors in the hippocampus (Herkenham *et al.*, 1990, 1991b). Both synthetic cannabinoid receptor agonists as well as the endogenous ligands anandamide (Terranova *et al.*, 1995) and 2-arachidonylglycerol (Stella *et al.*, 1997) have been reported to inhibit hippocampal long-term potientiation (LTP) of the Schaffer collateral commissural fiber-CA1 synapse, which represents a model for learning and memory, in a SR141716A-reversible manner.

Several of the well-known cannabinoid actions have been considered to be involved in the inhibition of LTP and impairment of memory. Thus, since CB1 receptors are located on presynaptic axon terminals, their activation causes inhibition of neurotransmitter release. It has been shown that cannabinoids are capable of inhibiting glutamate release in cultured rat hippocampal cells (Shen *et al.*, 1996) and acetylcholine release (Gifford *et al.*, 1997) as well as noradrenaline release (Schlicker *et al.*, 1997) in superfused hippocampal slices. The transduction mechanisms responsible for the decrease in neurotransmitter release and for the inhibition of LTP in the hippocampus include both the CB1 receptor-mediated inhibition of adenylate cyclase as well as the inhibition of N-type calcium channels (see Section 3.1.2). Although the modulation of neurotransmitter release, in particular inhibition of glutamate release, have been shown to inhibit the formation of LTP, it is also expected that they are involved in the neuroprotective effect of an acute administration of cannabinoids.

A putative neuroprotective action of an acute administration of cannabinoids, however, stands in marked contrast to the effects induced by chronic administration. While acute marijuana smoking seems to cause reversible impairment of memory, due to inhibition of glutamatergic, noradrenergic and cholinergic transmission, chronic marijuana smoking is expected to cause persistent memory and cognition deficiencies [for review see Court (1998)]. Already previously it has been reported that chronic cannabinoid administration to rats causes distinct morphological changes in the hippocampus, indicating a neurotoxic action (Scallet, 1991). Recently, Chan *et al.* (1998) confirmed the neurotoxicity of Δ^9-THC and presented intriguing evidence for the cellular mechanisms of action involved in this effect. The authors found that ca 50% of the cultivated hippocampal neurons which they have treated with 1 μM Δ^9-THC were killed within 6 days after exposure to Δ^9-THC. This concentration is comparable to the levels of Δ^9-THC measured in human plasma after consumption of a single marijuana cigarette (Chiang and Barnett, 1984). A higher Δ^9-THC concentration (10 μM) killed the neurons even within 3 hr. With respect to the Δ^9-THC-induced neuronal death, cultured hippocampal neurons are more sensitive than cultured cortical neurons (Chan *et al.*, 1998). Moreover these authors reported that the inhibition of the Δ^9-THC-induced hippocampal cell death was abolished by the CB1 receptor antagonist SR141716A, clearly indicating that this effect is mediated by the CB1 receptor and not by non-specific interactions with the membrane lipid phase. However, although Δ^9-THC neurotoxicity is mediated by the brain cannabinoid receptor, it appears to be independent from the CB1 receptor-mediated inhibition of adenylate cyclase or modulation of ion channels, since neither forskolin nor pertussis toxin pretreatment prevented the cell death. In contrast, Chan *et al.* observed that actinomycin D, an inhibitor of transcription, blocked the Δ^9-THC-induced toxicity, implying an activation of transcription-dependent cell death. Although the transcriptional events involved in the Δ^9-THC-induced neurotoxicity are not yet defined, there is evidence that CB1 receptors induce the expression of *Krox*-24 (Bouaboula *et al.*, 1995a) and that they increase the activity of nuclear factor-κB (Daaka *et al.*, 1997). Nuclear factor-κB activates the expression of several genes including tumor necrosis factor α (TNFα), which induces apoptotic cell death. Furthermore, the neurotoxic effect of Δ^9-THC is abrogated by vitamin E as well as by inhibitors of phospholipase A_2 and cyclooxygenase (Chan *et al.*, 1998), suggesting that CB1 receptor activation by Δ^9-THC may mediate stimulation of the cyclooxygenase pathway with generation of free radicals, which, in turn, can lead to lipid peroxidation and cell death. Finally, Chan *et al.* found increased DNA fragmentation and shrinkage of neuronal cell bodies and nuclei, hallmarks of neuronal apoptosis. The Δ^9-THC-induced apoptosis has recently also been observed outside the CNS (Zhu *et al.*, 1998). In time course studies examining DNA fragmenta-

tion and cell membrane integrity, these authors observed that fragmentation preceded membrane damage indicating that Δ^9-THC induces apoptosis rather than cell necrosis.

Taking these findings together, it appears that Δ^9-THC induces transcriptionally dependent cell death in the hippocampus, which may explain impairment of memory after chronic marijuana smoking (Court, 1998). In this context, endogenous cannabinoids could contribute to cell death in specific populations of neurons during neuronal development (Chan *et al*., 1998). Indeed, Berrendero *et al*. (1998) have detected cannabinoid receptor binding, mRNA expression and activation of signal transduction mechanisms in the fetal rat brain, which appear atypically distributed as compared with the adult brain.These findings support the view that the system constituted by these receptors and their putative endogenous ligands might play a role in specific molecular events of the brain development.

6. FUTURE DIRECTIONS

Cannabis does not appear to be a harmless recreational drug. Acute intoxication is characterized by euphoria, loss of short-term memory, stimulation of the senses, and impaired linear thinking. Depersonalization and panic attacks are adverse effects. Increased heart rate, reddened conjunctivae and immunosuppression are common peripheral effects. Chronically consumed high doses may cause impairment of cognitive abilities that are long-term, or even persistent.

The public debate centers upon the possible legalization of cannabis, at least for certain therapeutic purposes. Potential therapeutic applications of cannabinoids are the treatment of nausea and vomiting associated with chemotherapy as well as appetite stimulation in cancer and AIDS patients (Abood and Martin, 1992; Timpone *et al*., 1997; Voth and Schwartz, 1997). Despite the toxicity of Δ^9-THC described in the present review, evidence supports the selective use of pure Δ^9-THC preparations, applied orally at low doses, to treat nausea associated with cancer chemotherapy and to stimulate appetite [for review see Voth and Schwartz (1997)]. The evidence does not support the classification of crude marijuana as a prescribable medicine.

However, one should consider the potential immunosuppressive effects of cannabinoids mediated by CB2 receptors in the immune system, especially in patients with a comprised immune system. The problem of determining the therapeutic potential of cannabinoids is basically the same for any therapeutic purpose. The question is whether the desired effects are adequately selective compared with undesirable effects. Based on the recent progress in cannabinoid research, it is expected that receptor-selective agonist can eliminate many of the undesirable effects of Δ^9-THC by targeting only to one receptor. Problems in the development of CB1 receptor-selective drugs arise from the rapid development of tolerance, the induction of sedation and hypothermia, and the inhibition of locomotor activity. Since there is experimental evidence for an additive effect of opioids and low doses of cannabinoids (Welch and Stevens, 1992; Welch *et al*., 1995; Smith *et al*., 1998a), future efforts will focus on the use of cannabinoids as an adjunct therapy. As a recreational drug, marijuana poses dangers, particularly due to disturbance in the neurotransmitter release and, after chronic use, due to THC-induced cell death in such brain regions which are involved in cognition and memory. As a medical drug, cannabinoids should be available for patients who do not adequately respond to currently available therapies.

Not only cannabinoid receptor agonists are expected to be of clinical interest but also of partial agonists and antagonists. Partial agonists with high potency and low efficacy could have greater benefit to risk than full agonists. Antagonists could inhibit the endogenous background tone. It is conceivable that CB1 antagonists may possess a therapeutic potential for the treatment of cognitive disorders and memory deficits associated with aging or neurological diseases.

Although cannabinoid receptor research has made rapid progress due to the cloning of the cannabinoid receptors, discovery of endogenous ligand and availability of selective antagonist for each receptor, the elucidation of the possible pathophysiological roles of the cannabinoid CB1 and CB2 receptors and of their endogenous ligands require further research. Moreover, the pharmacological intervention in synthesis and breakdown of endogenous cannabinoids, in order to modulate their extracellular concentration, can provide potential therapeutic uses. Thus, future findings could well have a significant impact on the therapeutic exploitation of cannabinoid receptor agonists and antagonists.

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