Phytocannabinoids and Endocannabinoids

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Abstract: Progress in understanding the molecular mechanisms of cannabis action was made after discovery of cannabinoid receptors in the brain and the finding of endogenous metabolites with affinity to them. Activation of cannabinoid receptors on synaptic terminals results in regulation of ion channels, neurotransmitter release and synaptic plasticity. Neuromodulation of synapses by the cannabinoids is proving to have a wide range of functional effects, making them potential targets as medical preparations in a variety of illnesses, including some mental disorders and neurodegenerative illnesses. Cannabis contains a large amount of substances with affinity for the cannabinoid receptors. The endocannabinoids are a family of lipid neurotransmitters that engage the same membrane receptors targeted by tetrahydrocannabinol and that mediate retrograde signal from postsynaptic neurons to presynaptic ones. Discovery of endogenous cannabinoids and studies of the physiological functions of the cannabinoid system in the brain and body are producing a number of important findings about the role of membrane lipids and fatty acids in nerve signal transduction. Plant, endogenous and synthetic cannabinoids are using in these studies. The role of lipid membranes in the cannabinoid system follows from the fact that the source and supply of endogenous cannabinoids are derived from arachidonic acid, an important membrane constituent. The study of structure-activity relationships of molecules which influence the cannabinoid system in the brain and body is crucial in search of medical preparations with the therapeutic effects of the phytocannabinoids without the negative effects on cognitive function attributed to cannabis.

Keywords: Cannabis, endocannabinoids, synthetic cannabinoids, receptors, membranes, arachidonic acid, addiction, mental disorders.

1. HISTORY OF CANNABIS

Cannabis is one of the oldest multi-purpose commercial plants grown for its fibre, edible seeds and psychotropic substances [1, 2]. Historical background to cannabis and cannabis preparation as medicine gives Russo [3]. Most likely cannabis came from Central Asia and was first grown in China and then India. The first accounts of the medical use of cannabis come from China, India (Atharva Véda, 2000-1400 B.C.), Egypt, Syria, Persia and Tibet. While oral tradition dictates that the China's Emperor *Shen-Nung* prescribed cannabis in the 28th century B.C., this was not transcribed until the 2nd century C.E. Although the Greeks and Romans mainly used cannabis for fibre, they also used it as a medicine for a variety of purposes. Arabian physicians (e.g. Avicenna) also described the medical uses of cannabis. In medieval Europe, it was grown for its nutritious seeds but it was also mentioned in medical books. Thus, by the end of the 19th century, a fair degree of knowledge on the medical uses of cannabis in Europe and the United States existed and cannabis products were offered by major pharmacy companies for pain, whooping cough, asthma and as a soporific/ sedative.

The 20th century produced a large number of studies directed at the chemistry of the active substances in cannabis. However, the popularity of cannabis products in medicine virtually disappeared by the second half of the 20th century. In the late 1960s, a number of pharmaceutical companies attempted to develop cannabinoids with pharmacological effects and no psychotropic effects. They failed, and the social and political attitudes to the use of cannabinoids in medicine became increasingly conservative with sellers and users alike being prosecuted. Contemporary research has once again to prove to what degree and under which conditions it is possible to prescribe cannabinoids for various medical conditions.

A process for extracting the pharmacologically active substances from cannabis was patented as early as 1914 but new techniques for isolating the pure constituents were needed. Cannabinol (a weakly psychotropic constituent) and later cannabidiol (CBD; non-psychotropic constituent with other pharmacological effects) were successfully isolated and their chemical structure was determined in the first half of the 20th century. The synthesis and subsequent testing of various derivatives of these compounds confirmed that the tetrahydrocannabinols are mainly responsible for the psychotropic activity of the cannabis. Attempts were then made to isolate them and it was only demonstrated in the 1960s that Δ^9-tetrahydrocannabinol (Δ^9-THC, THC) is the main psychotropic constituent of the cannabis (Fig. **1**). The exact chemical structure of Δ^9-THC was described in 1964 [4] and its complete synthesis (or, more accurately, a mixture of its (–)- and (+)-optical isomers) was published in 1965 [5]. Demonstration of the existence of cannabinoid receptors in the central nervous system (CNS) in 1988 [6] and the discovery of the first endogenous cannabinoids [7, 8] were key events.

Interest in the potential medical uses of cannabinoids then re-appeared in the mid-1990s, largely as a result of studies showing that cannabis use helps patients cope with the pain and nausea of chemotherapy and that it lowers in-

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1874-4737/09 $55.00+.00

traocular tension in the case of glaucoma. THC derivatives with positive health effects but without psychotropic effects are the current aim of pharmaceutical preparations. Legally, some countries allow the medical use of cannabinoids. However, cannabis is currently listed as an illicit drug without medical use in most countries of the Euro-American world.

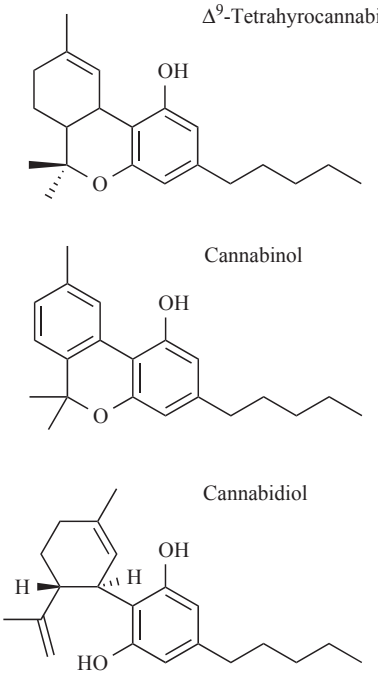

Fig. (1). Phytocannabinoids: psychotropic Δ^9-tetrahydrocannabinol (Δ^9-THC), weakly psychotropic cannabinol and non-psychotropic cannabidiol.

2. DEFINITIONS AND EXPLANATIONS

Cannabinoids are a group of substances originally found in cannabis plant (*Cannabis sativa* L) but they refer to any substance which is specifically recognized by the cannabinoid systems in the body [9, 10]. Currently, there are three general types of cannabinoids: herbal cannabinoids (phytocannabinoids); endogenous cannabinoids (endocannabinoids) found in the bodies of humans and other animals; and synthetic cannabinoids. The term tetrahydrocannabinol is usually used for the isomer, (–)-*trans*-Δ^9-tetrahydrocannabinol (dronabinol, formerly Δ^1-3,4-*trans*-tetrahydrocannabinol). The chemical structure is shown in Fig. (**1**).

Three basic herbal forms of cannabis predominate, and they are known by the Indian names: bhang (a seeded mixture of cannabis flowers, leaves, and stems, known as 'grass' in the USA), ganja (seedless unfertilized female flowering tops, termed sinsemilla, 'without seed', in North America), and charas (more commonly known as hashish in Arabic, a collection of cannabis resin *via* hand rubbing or sifting of trichomes from the cannabis flowers) [3]. Dried cannabis leaves and stems contain 1 – 3% of Δ^9-THC, flowering tops contain 3 – 20% of Δ^9-THC, and charas contains 5 – 20% of Δ^9-THC. Hash oil is an alcohol extract of cannabis and contains 20 – 60% of Δ^9-THC.

Phytocannabinoids can be administered by smoking, vaporizing, oral ingestion, intravenous injection, sublingual absorption, or rectal suppository. Transdermal THC delivery has been proved due to the advantages for therapeutic usage [11]. The dried mixture can be smoked like a cigarette (termed a joint) or in a pipe (bong) or wrapped in a tobacco leaf (blunt).

Psychotropic Δ^9-THC is present in most parts of the cannabis plant, with highest concentrations in sticky resin droplets produced by glands especially across the surface of the female inflorescence [12]. Seeds contain insignificant quantities of Δ^9-THC and hence, consuming them has no psychotropic effects. Cannabis resin is obtained by wiping it from flowers or "grinding" dried flowers and leaves through a number of sieves which removes dried resin particles; after pressing with a binding agent (e.g. fat) into a dense mass, yellow to dark brown hashish is obtained. Sinsemilla (marijuana without seeds) has been grown since the 1970s; i.e. only unpollinated female plants which produce more flowers are left to grow. Many varieties of cannabis, which vary in potency, have been produced, particularly in the Netherlands and California.

Cannabis contains at least 489 chemical constituents; 70 of which are phytocannabinoids. Several subclasses of cannabinoids have been identified [13]: 1) cannabigerol type; 2) cannabichromene type; 3) cannabidiol type; 4) (–)-Δ^9-*trans*-tetrahydrocannabinol type; 5) (–)-Δ^8-tetrahydrocannabinol type; 6) cannabicyclol type; 7) cannabielsoin type; 8) cannabinol type; 9) cannabinodiol-type; 10) cannabitriol type; and 11) miscellaneous types.

Δ^9-THC and CBD are the main constituents of cannabis with various pharmacological profiles. Δ^9-THC activates type-1 cannabinoid receptors, CB_1 (K_i = 25.1 nmol/L), and type-2 receptors, CB_2 (K_i = 35.2 nmol/L), while CBD has negligible affinity for these receptors (K_i = 2860 nmol/L for CB_2; there is no reported K_i for CB_1) [14]. Binding values differ due to experimental conditions and data from different laboratories may vary considerably, but the general trend is retained. Although CBD has low affinity for both CB_1 and CB_2 receptors, it displays high potency to antagonize cannabinoid receptor agonists [15]; rationale for combination of THC and CBD in pharmaceutical preparations was presented [16]. The degree of cannabis effects depends on content of Δ^9-THC. The pharmacological action of Δ^9-THC is stereoselective; only the (–)-isomer occurs in the nature. This is much more biologically active than its mirror (+)-isomer. Cannabinol and cannabichromene have slight Δ^9-THC-like effects [17-19] while CBD and cannabigerol are probably non-psychotropic cannabinoids. Cannabis also contains varying quantities of cannabinoid carboxylic acids which lack psychotropic effects and whose significance is based on the fact that they transform into an active form of THC after they are heated (during cooking or smoking). In contrast, Δ^9-THC may oxidize into non-active constituents when cannabis resin is stored.

3. PHYTOCANNABINOIDS

3.1. Effects and Mechanisms of Action

The effects of cannabinoids on emotional, cognitive and psychomotor function vary according to the experience of the user and his/her condition at the time of consuming the drug. The most marked psychological effects of THC can be divided into four groups: 1. affective (euphoria, cheerfulness), 2. sensoric (increased perception of external stimuli

and one's own body), 3. somatic (feeling of the body floating or falling), 4. cognitive (disturbed time perception, memory failure, troubles with concentration).

Cannabis also has a large number of physical effects, e.g. lowering of body temperature, reddened conjunctivae, reduced tear flow, decreased intraocular pressure, tachycardia, increased oxygen demands, vasodilatation, orthostatic or postural hypotension, hypertension, bronchodilation, hyposalivation, reduced bowel movement and delayed gastric emptying, changes in hormonal and immune system etc. [20].

A number of physiological effects (for instance, on blood pressure) can depend on experience and health status. The increase in resting pulse rate by as much as 60%, which occurs during the first thirty minutes of smoking marijuana, can be dangerous for people with cardiovascular disease for example. The effects of THC on the cardiovascular system are marked and mainly mediated by CB_1 receptors in blood cells and heart. However, recent studies provide support for existence of novel endothelial and cardiac receptors (distinct from CB_1 or CB_2) that mediate certain endocannabinoid-induced cardiovascular effects [21].

Cannabinoids have been reported to reduce intraocular pressure when given topically or systemically. Multiple lines of evidence suggest that endocannabinoids and CB_1 receptors in the retina play an important role in the regulation of intraocular pressure, and cannabinoid ligands may be of significant benefit in the treatment of glaucoma [22-24].

Cannabinoids are found to change the function of immune system cells. However, according to the World Health Organization [25] most of these changes are relatively small, completely reversible after cannabinoids removal, and produced at concentrations higher than those required for the psychoactivity of THC (more than 10 μmol/L *in vitro*, or more than 5 mg/kg *in vivo*).

Δ^9-THC is the most significant constituent of cannabis from a pharmacological and toxicological point of view. The action of low doses of Δ^9-THC is characterized by a mixture of depressive and stimulative effects on the CNS. Analogous to many other psychotropic substances, the acute effects of Δ^9-THC are well-known, but less is known about its long-term effects (see chapters 8. and 9.). Cannabis intoxication impairs cognitive processes. The neurochemical processes that lead to changes in the cognitive, affective and psychomotor function of marijuana smokers are still subject to on going research. Investigations of recovery of cognitive function with abstinence from cannabis have produced conflicting evidence, with some studies suggesting persistent deficits in specific cognitive functions beyond the period of acute intoxication [26-28]. However, many findings do not support the hypothesis that long-term heavy cannabis use causes irreversible cognitive deficits and these results would support the provision of clinical cannabis compounds to a greater number of patients in need [29-31].

The lipophilic properties of the cannabinoids firstly led to an assumption that they exert their effect *via* disturbance of the ordering of the lipid part of the cell membranes [32, 33]. The discovery of the endocannabinoid system and understanding cannabinoid psychoactivity with mouse genetic model evidenced that the CB_1 cannabinoid receptor is the principal molecular target of cannabinoids in the CNS [34-36]. Available findings show that CB_1 receptors in the brain are responsible for the psychotropic properties of the cannabinoids, including the reward effect, tolerance and physical addiction [37]. Cannabinoid signaling systems have been found in mammals, fish and invertebrates [38].

Δ^9-THC is an agonist for both CB_1 and CB_2 cannabinoid receptors. Both receptor types are coupled with G proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinases. Maximum inhibition of adenylate cyclase, and thus creation of cyclic adenosine monophosphate (cAMP), is achieved at Δ^9-THC concentrations of about 0.1 μmol/L [39]. cAMP is an important messenger for transfer of signals for the activation of various neurotransmitter receptors, and participates in regulating cellular functions *via* activation of A-type protein kinases (PKA). Δ^9-THC is only a partial agonist of CB_1 receptors as it is not able to trigger full activation of the receptor, unlike synthetic cannabinoids CP 55,940 and WIN 55,212-2. Further, CB_1 receptors are connected *via* $G_{i/o}$ proteins to ion channels, negatively with N-type and P/Q-type calcium channels, and positively with various types of potassium channels [40]. Inhibition of calcium channels can explain cannabinoid induced reduction of release of neurotransmitters, such as glutamate, γ-aminobutyric acid (GABA), acetylcholine, norepinephrine and others, from presynaptic terminals.

Understanding the biochemical effects of cannabinoids involves not only processes induced by direct activation of cannabinoid receptors but also downstream targets regulated by chronic administration of a drug; for instance, the serotonergic system is involved in many processes which are also influenced by the use of cannabinoids [41, 42].

CB_1 receptors are found predominantly in the brain but they also occur in the spinal cord and peripheral nervous system. They are especially located in the area of synapses, and one of their functions involves modulation of neurotransmitter release. CB_2 receptors are found in the immune system but they have also been found in inflamed brain where their expression in microglia is modulated by cytokines [43]. The presence and function of CB_2 receptors in healthy brain has been described [44] but is still controversial.

CB_1 receptors are abundant metabotropic receptors in the brain and are involved in multiple important physiological and behavioral events. The density of the CB_1 receptor in the brain is comparable to other G protein-coupled receptors (GPCRs), such the Mu opioid receptors and dopamine D2 receptors. CB_1 receptors are especially numerous in substantia nigra > globus pallidus > dentate gyrus > hippocampus > cerebral cortex > putamen > caudate > cerebellum > amygdala > thalamus = hypothalamus. Low concentrations were found in brain stem nuclei controlling respiration [14, 45-47].

CB_2 receptors probably participate in the immunosuppressive and antinociceptive effects of cannabinoids [48, 49]. It was found that unsaturated fatty acid *N*-alkylamides (alkamides) from purple coneflower (*Echinacea* spp.) constitute a new class of cannabinomimetics, which specifically engage and activate the CB_2 receptors [50, 51]. Alkamides

binding to CB_2 receptors is considered as a possible molecular mode of their immunomodulatory effects.

3.2. Pharmacokinetics

Depending on the route of administration, there are marked differences in THC resorption and metabolism and its accompanying manifestations. Natural cannabinoids must be decarboxylated (e.g. through heating) before ingestion. Smoking is a particularly effective manner of getting Δ^9-THC into the brain. After inhaling, Δ^9-THC is quickly resorbed in the blood flow and distributed [52]. 15 – 50% of Δ^9-THC from a marijuana cigarette reaches the body's circulation system, while the losses in experienced smokers are up to a half of those in random smokers. Subjective feelings are perceived within seconds or minutes after smoking. They reach a maximum after 30 minutes and last for 2 to 3 hours (weaker effects are perceived for an even longer time). After oral consumption, the effects come after 0.5 - 2 hours, are more permanent and last for 5 to 8 hours (or even longer when larger doses are consumed). Intravenous application of THC is difficult because it does not dissolve in water. In oral consumption, THC resorption is better when it is consumed with fat. The psychotropic threshold is 0.03-0.1 mg/kg when inhaled, 0.2-0.3 mg/kg when consumed orally (with a lipophilic carrier), and 0.01-0.02 mg/kg when injected [53]. While the oral route of administration achieves only limited blood concentrations, significant transient psychotic symptoms may occur [54].

THC and its metabolites penetrate tissues quickly. As THC is highly soluble in fats, it especially accumulates in body fat. The apparent distribution volume of THC is about 10 L/kg. In plasma, most THC is bound to lipoproteins, albumin and erythrocytes. The free fraction is only approximately 3%. Slow release of THC from fatty tissues to blood does not normally lead to concentrations causing psychological effects. Regular cannabis use leads to marked accumulation of THC in fatty tissues.

A plasma THC concentration of about 0.1 μmol/L is sufficient for psychotropic effects [55, 56]. The main metabolizing organ is the liver, although the lungs and the intestines also participate in biotransformation of Δ^9-THC. 11-Hydroxy-Δ^9-THC (11-OH-THC) is the main active metabolite; effective metabolism in liver then changes 11-OH-THC to non-psychotropic 11-nor-Δ^9-THC-9-carboxylic acid (THC-COOH) and other metabolites (Fig. **2**). Plasma levels of Δ^9-THC of smoked marijuana increase very quickly and reach a maximum (usually tens of ng/mL) before the end of smoking and then quickly decrease due to distribution to highly vascular tissues (α elimination phase), after which slower distribution to fatty tissue follows (β elimination phase). Clearance is 760 to 1200 mL/min, final plasma elimination half-life is reported to be 1-4 days. Complete elimination of a single dose of Δ^9-THC may last for up to five weeks but THC metabolites have been found in urine of chronic smokers 80 days after the last dose. 11-Nor-Δ^9-THC-9-carboxylic acid-glucuronide (THC-COOHglu) is the main THC metabolite in urine [2, 57, 58].

4. ENDOCANNABINOIDS

4.1. Effects and Mechanisms of Action

Endogenous cannabinoids (endocannabinoids) are lipophilic signal molecules which are synthesized *de novo* from membrane phospholipids and released in response to postsynaptic depolarization or activation of metabotropic glutamate receptors. They meet the criteria for listing as neurotransmitters, but unlike classic neurotransmitters they are not synthesized in the cytosol of neuron and are not stored in

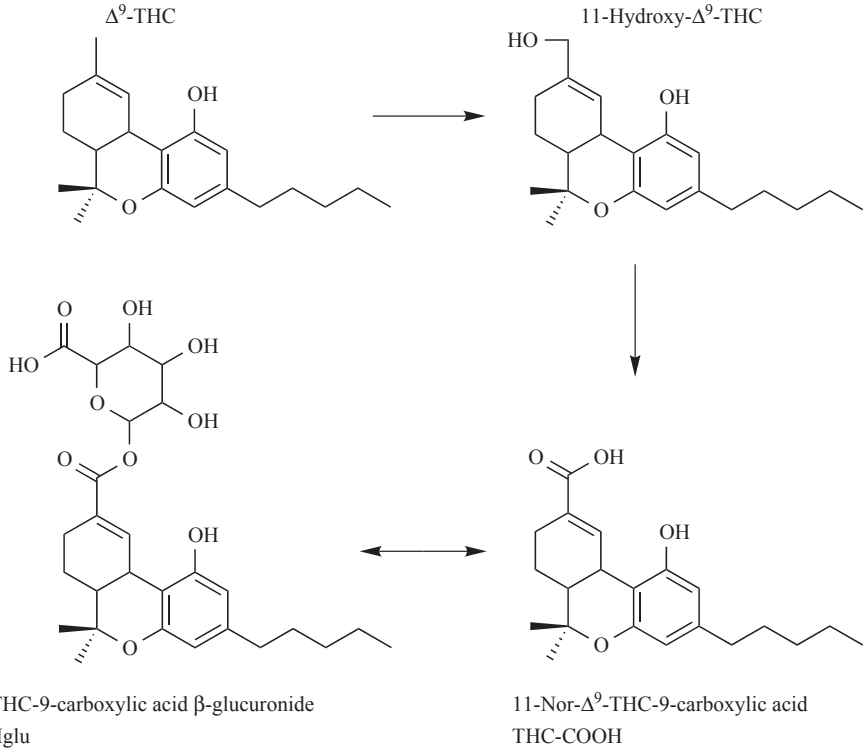

Fig. (2). Metabolism of Δ^9-tetrahydrocannabinol (Δ^9-THC).

synaptic vesicles. Endocannabinoids are widely distributed in the brain and throughout the body. *sn*-2-Arachidonoylglycerol (2-AG) [8, 59] is the most prevalent endogenous ligand of cannabinoid receptors in mammals, and anandamide (*N*-arachidonoylethanolamide, AEA) [7] has been the most explored (Fig. **3**). Additional putative endocannabinoids are noladin ether (2-arachidonoylglycerylether) [60], virodhamine (*O*-arachidonoylethanolamine) [61], *N*-arachidonoyldopamine (NADA) [62] and others [47, 63-65].

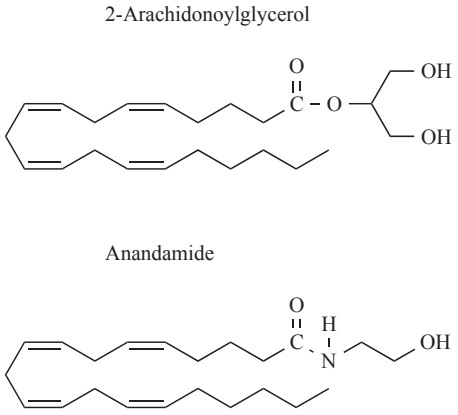

Fig. (3). Endogenous cannabinoids: 2-arachidonoylglycerol and anandamide.

Anandamide and 2-AG are synthesized in brain, in response to increased calcium concentrations. Anandamide exhibits binding affinity to both human cannabinoid receptors CB_1 (K_i = 239 nmol/L; partial agonist) and CB_2 (440 nmol/L) [14]. Anandamide concentrations in various brain areas were found to be less than 100 pmol/g tissue [66, 67], although higher amounts have been measured (> 1 nmol/g tissue) [68, 69] and intracellular concentrations can be much higher [70]. Mechanisms of cellular uptake of anandamide are not completely known to date but it is assumed that they involve free diffusion across membranes as well as carrier proteins [71]. The likelihood of specific processes involved in transfer of endocannabinoids *via* membrane, follows on from the fact that they are released from cells after synthesis and transferred back to them before enzymatic hydrolysis. Analogically to anandamide, 2-AG also has binding affinity to human CB_1 (3424 nmol/L; full agonist) as well as CB_2 (1194 nmol/L) receptors [14]. In the brain, it is found in concentrations of about 4 nmol/g, i.e. markedly higher than anandamide [59, 72].

Anandamide binds competitively to brain cannabinoid receptors, inhibits adenylate cyclase, inhibits voltage-dependent calcium channels and exhibits many, though not all, of the pharmacological and behavioral effects of THC [73-75]. Unlike phytocannabinoids, it is quickly inactivated, and so its behavioral effects are difficult to measure. Increase in neurochemical effects of anandamide can be observed at higher doses [76], or when its metabolism by the fatty acid amide hydrolase (FAAH) is inhibited [77].

CB_1 receptor positive terminals target both the dentritic and somatic surface of neurons. These receptors are abundant on GABAergic neurons [78]. Receptor distribution along the axonal membrane was determined using antibodies against the CB_1 receptor and it was shown that they are rare in the synaptic active zone, but are enriched in the perisynaptic annulus, where they can influence calcium channels [79]. It has been confirmed in recent years that endocannabinoids mediate retrograde signal from postsynaptic neurons to presynaptic ones (Fig. **4**), i.e. induced release of many neurotransmitters in brain can be inhibited by activation of presynaptic CB_1 receptors [2, 40, 64, 80, 81]. Inhibition by endocannabinoids lasts for up to tens of seconds [82], i.e. much longer that inhibition caused by release of GABA and much shorter than necessary for change in synaptic strength induced by some form of synaptic plasticity. Chief functions of cannabinoid receptors involve suppression of GABA release, glutamate release and reuptake [83, 84] and they also influence the release of many other neurotransmitters [85]. However, endocannabinoid signaling may play roles other than reducing neurotransmitter release from axon terminals, because density of CB_1 receptors was found significantly higher on preterminal axons than on synaptic terminals [79].

It is apparent that by retrograde signaling endocannabinoids play important role in modulation of synaptic plasticity in the CNS. Endocannabinoid-mediated short-term synaptic plasticity includes both depolarization-induced suppression of inhibition, which is due to reduction of GABA release, and depolarization-induced suppression of excitation, which results from inhibition of glutamate release. It is now clear that presynaptic depression is the major physiological role for CB_1 receptor. There is also a key role of the endocannabinoid system in one form of long-term synaptic depression that involves a long-lasting decrease in neurotransmitter release [86-90].

Cannabinoid-induced reduction of the production of cAMP and activation of mitogen-activated protein kinases (MAPK) cascade leads to changes in phosphorylation and so also in the function of many cellular molecules, including several transcription factors. Information about activation of postsynaptic cannabinoid receptors can therefore be transferred up to changes in gene expression and thus to modulation of synaptic plasticity. Phosphorylated transcription factor CREB (cAMP response element-binding protein) facilitates increased production of brain-derived neurotrophic factor (BDNF), which activates trkB receptors and increases gene expression of a series of cellular molecules. This permits the neuron to form new synaptic connections, memory traces etc. [91, 92]. We may hypothesize that reduction in cAMP concentrations induced by activation of postsynaptic cannabinoid receptors leads to lower CREB phosphorylation, and subsequently reduction in neuron plasticity in the hippocampus. On the other hand, inhibition of nitric-oxide synthase and PKA observed after activation of CB_1 receptors may protect neurons against neurotoxic damage [93, 94]. Cannabinoids exert different effects on gene expression in different brain areas, e.g. increased expression of BDNF was reported in nucleus accumbens and in other specific brain regions associated with reward after long-term administration of THC to rats [95].

The physiological functions of the cannabinoid system are very complex and involve motor coordination (basal ganglia), memory (hippocampus), appetite, pain modulation (hindbrain), neuroprotection, and maintenance of homeostasis, among others [24, 96, 97]. Other subtypes of cannabinoid

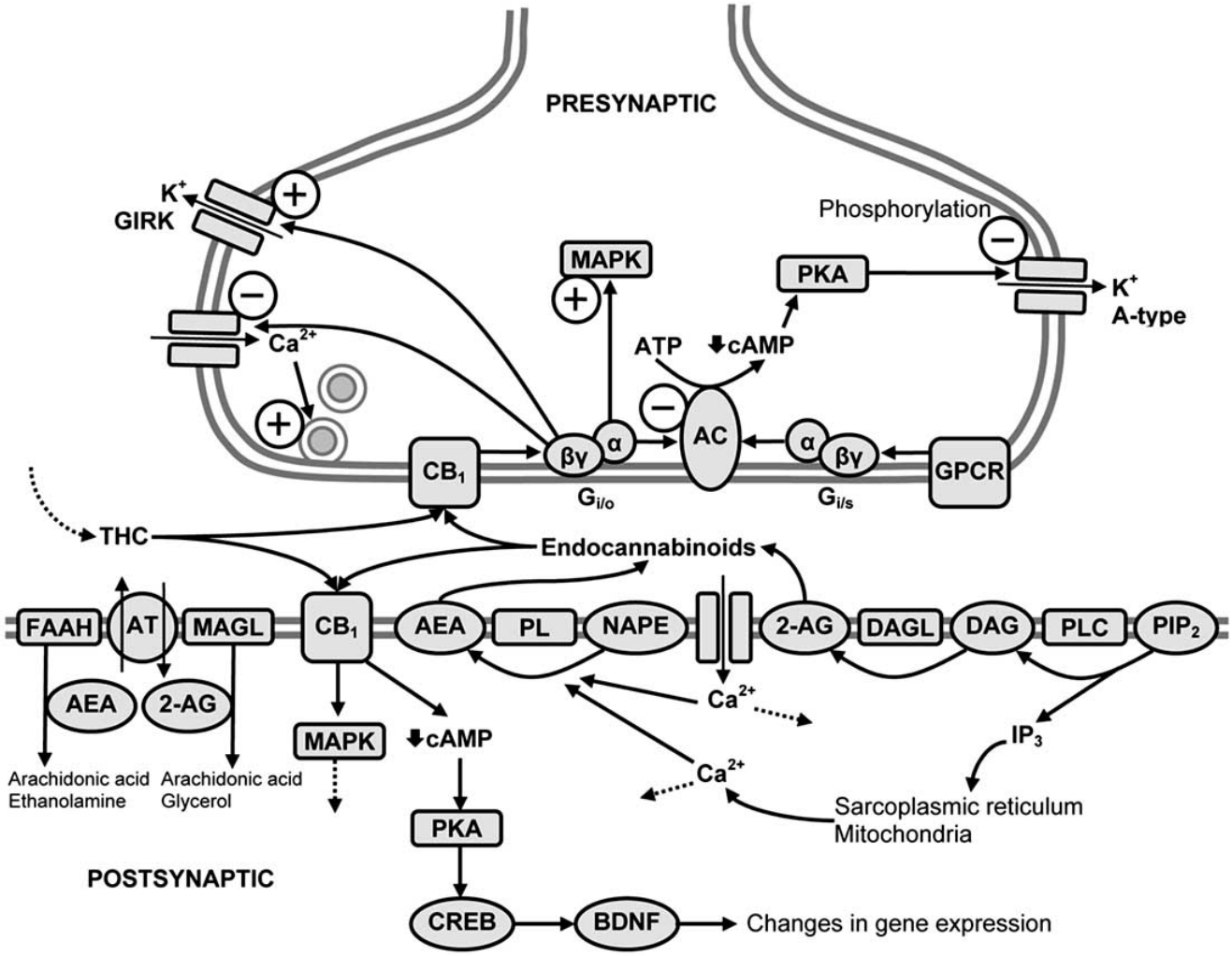

Fig. (4). Mechanisms of action of cannabinoids. A large transient increase in intracellular calcium by Ca^{2+} release from intracellular store (by activation of the inositol-1,4,5-triphosphate system), inflow of Ca^{2+} *via* voltage dependent ion channels or through internal ion channel of activated receptors is required for the synthesis of endocannabinoids. This is assumed to induce stimulation of phospholipases (PL) or hydrolases, that catalyses hydrolysis of *N*-arachidonoyl phosphatidylethanolamine (NAPE) and anandamide (AEA) is formed. Phospholipase C (PLC) catalyses hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP_2) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3) and diacylglycerol lipase (DAGL) then catalyses the production of 2-arachidonoylglycerol (2-AG). Newly created endogenous cannabinoids or externally supplied tetrahydrocannabinols (THC) activate cannabinoid receptors CB_1 localized in the presynaptic or postsynaptic membrane. Anandamide is removed from the extracellular environment by a specific membrane carrier (AT) or free diffusion and is hydrolyzed in the cell to arachidonic acid and ethanolamine by means of a membrane enzyme, fatty acid amide hydrolase (FAAH). AT can also carry 2-AG, which is then hydrolyzed with monoacylglycerol lipase (MAGL) to arachidonic acid and glycerol. Activation of the CB_1 receptor in the presynaptic nerve terminal activates $G_{i/o}$ proteins, which stimulate mitogen-activated protein kinases (MAPK) and inhibit adenylate cyclase (AC). This reduces production of cyclic adenosine monophosphate (cAMP). Further, βγ subunits of G proteins activated by CB_1 receptors inhibit voltage dependent calcium channels and stimulate G protein-activated inwardly rectifying K^+-channels (GIRK). Inhibition of the uptake of calcium to presynaptic terminal causes reduced release of various neurotransmitters. Lowered cAMP concentration reduces activation of type A protein kinase (PKA), which *inter alia* causes lower phosphorylation of voltage-gated potassium channels (A-type) and subsequent further increase in potassium ion outflow. Activation of the CB_1 receptor may also influence the function of many other receptors which are coupled to G proteins (GPCR) and activate or inhibit AC. Postsynaptic levels of cAMP can affect synaptic plasticity through changes in activity of PKA, phosphorylation of specific transcription factor (CREB) and gene expression of the brain-derived neurotrophic factor (BDNF) and other cellular compounds.

receptors are involved in these processes, as well as TRPV1 receptor (vanilloid/capsaicin receptor; anandamide was identified as the first endovanilloid) [98, 99]. From the point of view of behavioral mechanisms of action, it is assumed that the functional role of endocannabinoids in the brain consists in tempering excessive arousal and excessive cognitive functions and also in increasing responses to novelty and as such, increasing perceptual function and facilitating hedonic processes [2].

Many of the pharmacological effects of cannabinoids can be explained by their interactions with neuromodulators and

neurotransmitter systems; inhibition of release of neurotransmitters by agonists of CB_1 receptors has been described for acetylcholine, dopamine, GABA, histamine, serotonin, glutamate, noradrenalin, prostaglandins and opioid peptides. However, in interpreting the effects of cannabinoids, it should be recognized that inhibition of neurotransmitter release does not mean a general reduction in neurotransmitter activity because some neurotransmitters activate receptors which inhibit signal transduction. Functional interactions of the cannabinoid system with the dopaminergic and the opioid systems are probably the most significant for the onset of the reward effect, tolerance and physical dependence to cannabinoids [37]. CB_1 antagonism might be useful in treating drug dependence [100].

Most effects of cannabinoids are mediated by cannabinoid receptors in CNS [81], but there are also effects which are at least partly independent of CB receptors, e.g. neuroprotective effects in ischemia and hypoxia [101]. Moreover, it was discovered that anandamide may not be producing all of its effects by a direct interaction with the CB_1 receptor [75]. Tests with CB_1 receptor knockout mice provided pharmacological and biochemical evidence for the existence of non-CB_1, non-CB_2 G protein-coupled brain receptors for anandamide that may be partly responsible for some of the effects of this substance on nociception and motor behavior in mice [102]. It seems that cannabinoids activate at least five distinct cannabinoid receptors and an additional endocannabinoid signaling system that involves palmitoylethanolamide may exist [103]. Convincing *in vitro* evidences were presented that the orphan GPCR, GPR55, is a cannabinoid receptor [104-107]. It is assumed that some actions of endogenous cannabinoids are mediated by their metabolites or *via* non-receptor mechanisms or hitherto unknown cannabinoid receptors.

4.2. Biosynthesis and Inactivation

Anandamide, 2-AG and several other endogenous cannabinoids (Fig. **3**) are derived from arachidonic acid (all-*cis* 5,8,11,14-eicosatetraenoic acid, 20:4), which is one of the unsaturated fatty acids found in the phospholipids of the cell membrane. Arachidonic acid is an unsaturated C_{20} fatty acid with four double (unconjugated) bonds; the double bonds on carbon $C_{(14)}$ is located six carbon atoms away from the end carbon (it is an n-6 fatty acid). In humans, arachidonic acid is a precursor of prostaglandins, prostacyclines, thromboxanes and leukotrienes [108]. Arachidonic acid forms up to 10 % of all fatty acids in the brain. Arachidonic acid is stored in membranes and binds especially to glycerol carbon $C_{(2)}$ (*sn*-2) in phospholipids, and is released *via* 1. hydrolysis of these molecules by phospholipase A_2 (PLA_2); 2. combined action of phospholipase C (PLC), diacylglycerol kinase and PLA_2; 3. hydrolysis by diacylglycerol lipase. Most of the arachidonic acid is incorporated in phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine.

Anandamide and 2-AG probably are not synthesized to be stored, but on demand, in response to postsynaptic depolarization or receptor activation [82, 109]. The details of its synthesis are still unknown but it seems that anandamide can be stored in membranes as *N*-arachidonoyl phosphatidylethanolamine (NAPE), i.e. esterified on the third carbon of the *sn*-glycerol-3-phosphate [110]. This anandamide precursor only forms approximately 0.1% of all *N*-acyl ethanolamine phospholipids in neurons [111]. An *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) was identified as a candidate enzyme involved in biosynthesis *N*-acyl ethanolamines (NAEs) that include anandamide [112]. However, an alternative biochemical pathway for NAE production was revealed in genetic studies using mice with a targeted disruption of the NAPE-PLD gene. It was shown that NAPE-PLD is a principal regulator of long chain saturated NAEs in the nervous system, but biosynthesis of polyunsaturated NAEs, including anandamide, appears to be predominately controlled by other enzymes [113]. So, the several, parallel pathways of anandamide release from NAPE have been studied [99, 112, 114-117, 126]: 1. hydrolysis of NAPE by a phospholipase D (NAPE-PLD); 2. hydrolysis of NAPE by phospholipase A_1 (PLA_1) or A_2 to *N*-arachidonoyl lysophosphatidylethanolamine with subsequent anandamide release *via* lysophospholipase D; 3. the sequential double deacylation of NAPE by α,β-hydrolase 4 and the subsequent cleavage of glycerolphosphoanandamide to yield anandamide; and 4. phospholipase C dependent hydrolysis of NAPE to yield phosphoanandamide, which is subsequently dephosphorylated by phosphatases, including the tyrosine phosphatase and the inositol-5'-phosphatase (Fig. **5**).

It is generally assumed that in the brain, as well as in other tissues, arachidonic acid is esterified to the *sn*-2 position of phospholipids with saturated or monounsaturated fatty acids, such as the oleic acid (18:1), palmitic acid (16:0) or stearic acid (18:0) in the *sn*-1 position [118]. This is a little contrary to the substrate specificity of *N*-acyltransferase, which catalyses the biosynthesis of NAPE in the brain by transferring the arachidonate group from the *sn*-1 carbon of phospholipids to the amino group of phosphatidylethanolamine. However, it has been confirmed that *sn*-1 arachidonoyl phospholipids are present in the brain, and they form approximately 0.5% of all phospholipids [110, 119].

Anandamide production as well as NAPE synthesis can take place in parallel and they are initiated by an increase in intracellular Ca^{2+}. Direct anandamide synthesis catalyzed by FAAH is possible at high (non-physiological) concentrations of arachidonic acid and ethanolamine although this synthesis probably has no significance in the physiological production of anandamide.

2-AG biosynthesis in the brain is also initiated by neuronal activity accompanied by an increase in intracellular concentrations of Ca^{2+}. Unlike anandamide, 2-AG synthesis involves the same enzyme cascade as that which is involved in the production of second messengers, inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerole (DAG). 2-AG can be produced from DAG after hydrolysis of membrane lipids containing arachidonic acid. Inositol phospholipids or phosphatidic acids are probably the main sources of 2-AG. At the same time, 2-arachidonoyl-*sn*-glycero-3-phophate which forms a significant part (5.4%) of lysophosphatidic acids in the brain can be dephosphorylated to 2-AG by specific phosphatase [120]. Conversely, 2-AG can be converted to 2-arachidonoyl-*sn*-glycero-3-phosphate with the help of monoacylglycerol kinase [121]. For this reason, it is assumed that 2-AG biosynthesis is possible *via* two main pathways [47] (Fig. **6**):

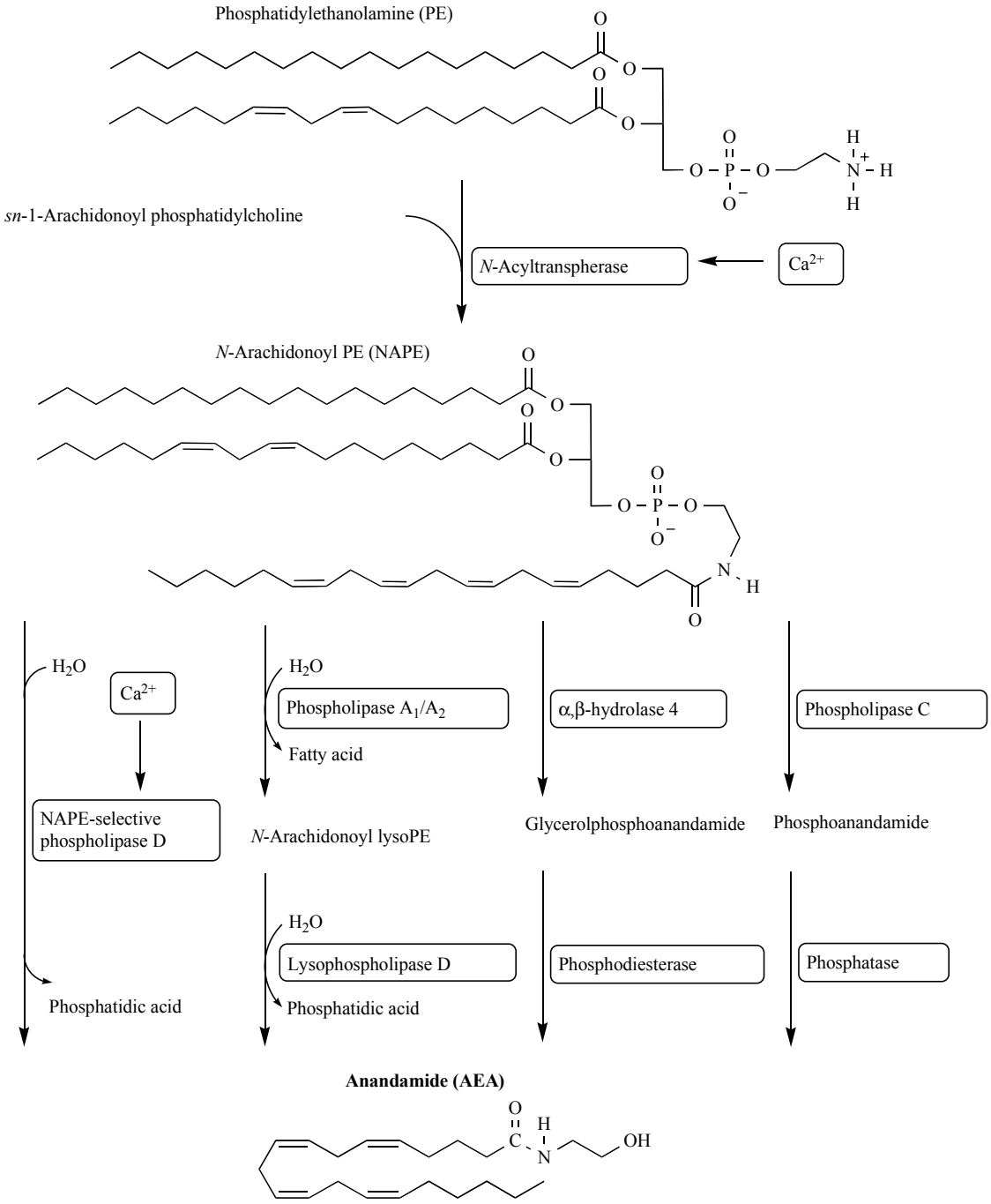

Fig. (5). Biosynthesis of anandamide.

1. Hydrolysis of membrane phospholipids by means of activated PLC produces DAG (usually 1-stearoyl-2-arachidonoyl-*sn*-glycerol), which is then converted to 2-AG by means of 1,2-diacylglycerol lipase (DAGL);
2. PLA_1 produces a lysophospholipid, which may then be hydrolyzed to a) 2-AG by means of lysophospholipase C, b) lysophosphatidic acid by means of lysophospholipase D and then dephosphorylated to 2-AG.

Like other neurotransmitters, endocannabinoids are quickly inactivated after production and release (Fig. **7**). Anandamide is transported back to neurons and glia and then hydrolyzed into arachidonic acid and ethanolamine by means of FAAH or other intracellular amidase [122, 123]. In addition to its hydrolysis by FAAH, anandamide is metabolized by cyclooxygenase-2 (COX-2), lipoxygenase (LOX) and cytochrome P450 [99, 124].

2-AG is transported to cells by a specific 2-AG transporter, *via* putative anandamide transporter, or by simple diffusion [125]. The assumption that FAAH is also responsible for the 2-AG elimination, which follows from the fact that FAAH catalyses hydrolysis of 2-AG *in vitro*, has not been confirmed. It is likely that monoacylglycerol lipase (MAGL), which converts 2- and 1- monoglycerides into fatty acid and glycerol, is responsible for the hydrolysis most

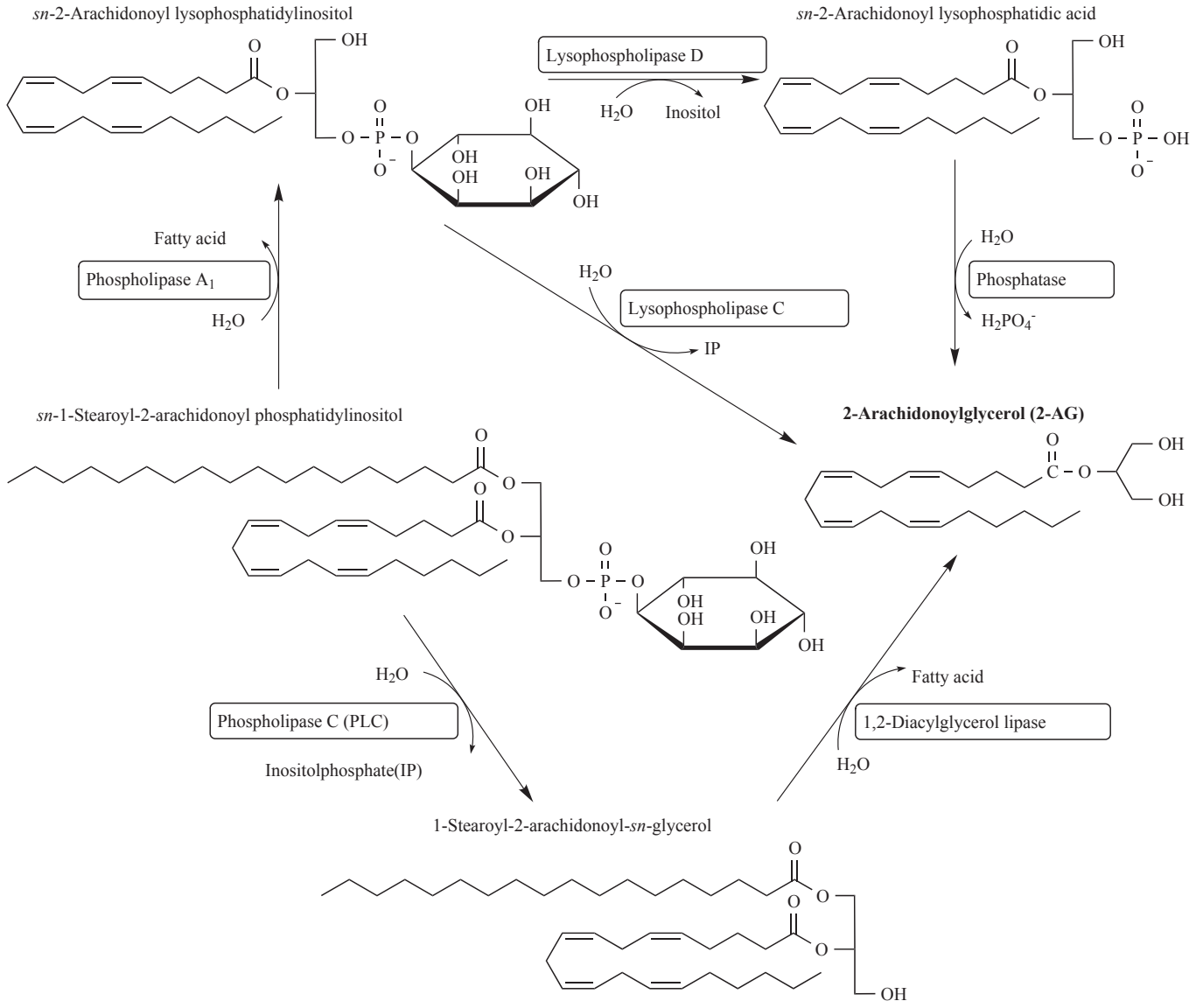

Fig. (6). Biosynthesis of 2-arachidonoylglycerol (2-AG).

of 2-AG in the brain [126, 127]. In addition to neuronal MAGL, other enzymatic activities have been identified such as a novel, pharmacologically distinct, MAGL activity expressed by microglial cells [128] or other uncharacterized enzymes [127]. Moreover, phosphorylation of 2-AG by monoacylglycerol kinase may participate in 2-AG inactivation [121].

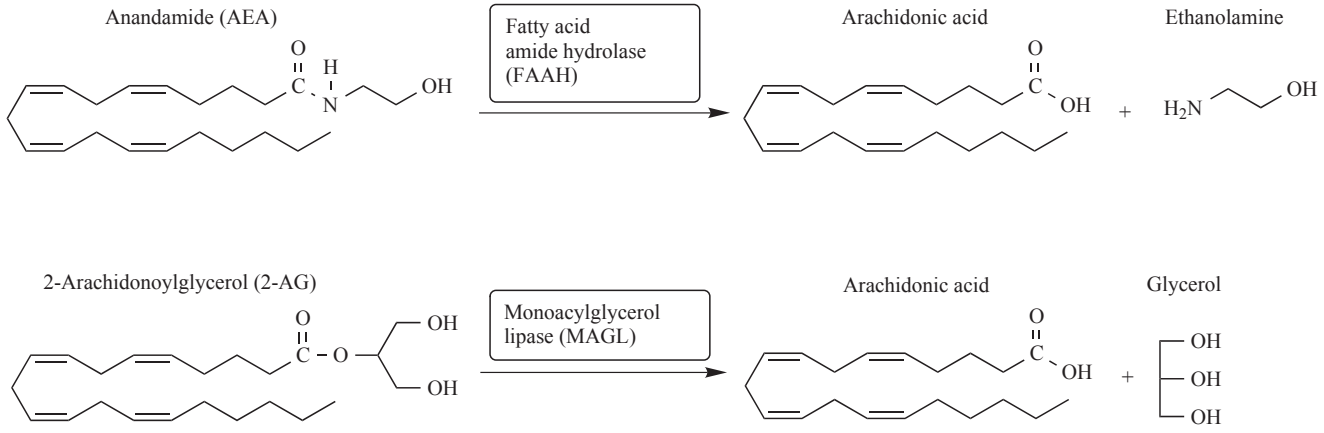

Fig. (7). Catabolism of endocannabinoids.

A similar system of inactivation has long been known for monoamine neurotransmitters, such as serotonin, norepinephrine and dopamine, which are transported inside the cell by specific transporters and then metabolized by mitochondrial monoamine oxidase. Therefore, just as serotonin, noradrenalin and dopamine reuptake inhibitors or inhibitors of monoamine oxidase are effective antidepressants, FAAH or the cannabinoid carrier may be target sites of the primary effects of pharmaceutical preparations which interfere with the cannabinoid system in the brain [129].

The different synthesis, transport and metabolism of anandamide and 2-AG indicate that there are specific regulation mechanisms for these substances. The most recent findings confirmed that endocannabinoid system is not to be considered to be made of interchangeable molecules with similar physiological roles. Anandamide directly inhibits the synthesis of 2-AG [130] and it was shown that elevation of anandamide concentrations in the striatum reduced the physiological effects of 2-AG on GABAergic transmission [131]. So, the metabolic and functional interactions between endocannabinoids might be involved in the control of synaptic transmission.

5. SYNTHETIC CANNABINOIDS

A series of extremely potent cannabinoid receptor agonists and cannabinoid receptor antagonists/inverse agonists have been synthesized. Until the early 1990s, all the compounds known act as cannabimimetics were structurally derived from THC. Classification of cannabinoid receptor ligands into classical, nonclassical, aminoalkyindoles, and eicosanoids have been used [9, 132]; however, the new potent ligands of cannabinoid receptors were designed and synthesized whose chemical definition encompasses a variety of chemical classes (1,5-diarylpyrazoles, quinolines and arylsulphonamides, and additional compounds) which are no longer related to natural or endogenous cannabinoids [9, 10, 132-134]. They have assisted in the detailed localization of cannabinoid receptors and characterization of their pharmacological properties. Attention has been paid to CB_1 receptors particularly; however, a number of selective CB_2 receptor ligands were synthesized due to involvement of CB_2 receptors in signal transduction in immune system [133, 135]. There is good correlation between CB_1 receptor affinity and the *in vivo* activity of cannabinoids [136]. The pharmacological effects of the prototypal cannabinoid agonist Δ^9-THC can be blocked by the synthetic cannabinoid antagonist (e.g. SR141716A) [137]. So, synthetic cannabinoids are particularly useful in experiments to determine the relationship between the structure and activity and they facilitate behavioral and neurobiological research with cannabinoids.

5.1. Cannabinoid Receptor Agonists

Classical cannabinoids are tricyclic terpenoid derivatives bearing a benzopyran moiety. The most investigated synthetic analogs of classical cannabinoids (Δ^9-THC, Δ^8-THC) have been 11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210). HU-210 has affinities for CB_1 and CB_2 receptors that exceed those of these other cannabinoids. As a result, it is a particularly potent cannabinoid receptor agonist. CB_2 selective cannabinoid receptor agonists are L-759633, JWH-051, JWH-133, and many others [133].

Analogs lacking the dihydropyran ring of THC (nonclassical cannabinoids) were synthesized, e.g. bicyclic CP47497 or CP 55,940, and tricyclic CP55244. The best known compound is CP 55,940 (Fig. **8**); it binds to CB_1 and CB_2 receptors with similar affinity and displays higher activity *in vivo* as compared with Δ^9-THC [9]. Binding assay for cannabinoid receptors was developed using tritiated CP 55,940 [6]. Using quantitative autoradiography, the distribution of cannabinoid receptors was reported in a variety of mammals [46]. HU-308 is non-classical cannabinoid CB_2-selective receptor agonists.

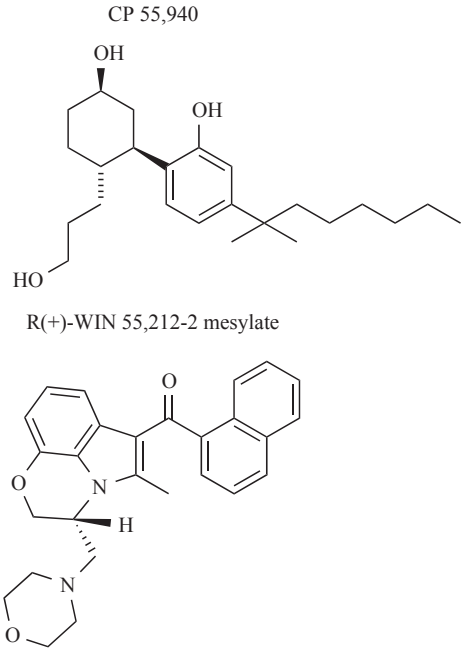

Fig. (8). Synthetic cannabinoids: CP 55,940 and WIN 55,212-2.

A new family of aminoalkyindoles possessing cannabimimetic properties was reported in the early 1990s [138]. *R*-(+)-WIN55,212 (Fig. **8**) is the most highly studied compound of this class. It displays high affinity for CB_1 and CB_2 receptors and shows higher relative intrinsic activity as compared with Δ^9-THC. In view of the major structural differences that exist between the aminoalkylindoles and other cannabinoid receptor agonists, it is not at all surprising that there is evidence that the WIN 55,212-2 binds differently to cannabinoid CB_1 receptors than members of other chemical groups of cannabinoids [139].

Hybrid cannabinoids is new class of analogs incorporated all of the structural features of both classical and nonclassical cannabinoids. Additionally the various head and tail modified anandamide analogs possess cannabinoid receptor agonist properties [140], e.g. CB_1-selective agonists *R*-(+)-methanandamide, arachidonoyl-2'-chloroethylamide (ACEA), and arachidonoylcyclopropylamide (ACPA).

5.2. Cannabinoid Receptor Antagonists/Inverse Agonists

The first CB_1 receptor antagonist SR141716A (rimonabant) [141] is currently tested for treatment of psychotic disorders and obesity. Another notable CB_1 receptor selective antagonist is SR147778 [142] or LY320135 [143] The notable CB_2 receptor antagonist/inverse agonist is SR144528 [144]. AM630 (iodopravadoline) is the first CB_2-selective

aminoalkylindole antagonists [145]. Without selective agonists and antagonists/inverse agonists, our knowledge of the roles played by the cannabinoid system in the body would be very much more rudimentary [123].

6. CELL MEMBRANES AND CANNABINOIDS

Cannabinoids are substances which like many other lipophilic molecules, are incorporated into the cell membrane lipid bilayer [146]. It is unknown whether accumulation in the lipid part of biological membranes is or is not linked so some of their side effects. However, it is known that the protein-lipid-lipophilic molecule interactions can influence the function of many membrane systems which participate in cellular functions [147, 148], including nerve signal transduction. Although membrane lipids are not specific target molecules of cannabinoids, they can play an important role in the mechanism of their effects, not only as a source of endogenous cannabinoids but also as molecules which determine the pharmacokinetics of cannabinoids and facilitate or modulate interactions of cannabinoids with specific protein binding sites [149-151]. The lipophilic nature of the agonists and antagonists of cannabinoid receptors suggests that the specific receptor binding site is localized in the hydrophobic core of the membrane.

Biological membranes are complex systems which can be taken schematically as lipid bilayers forming the basis for distribution and function of integral or peripheral proteins and these support the many intricate functions of the membrane. Membranes maintain the ion and metabolic gradients necessary for most cell functions, including nerve signal transduction. Glycerophospholipids are the main lipid constituents of cell membranes; they contain a glycerol core with fatty acids. Sphingolipids are also plentiful in brain, especially sphingomyelins and galactocerebrosides. Cholesterol is also an important integral constituent of plasma membranes. Membrane lipids do not only form the structural basis of membranes, they are also substrates of phospholipases and modulators of the function of many membrane proteins. For instance, signal transduction mechanisms which involve receptors, ion channels, enzymes, transporters and pumps are often regulated by membrane lipids and cholesterol, alone and arranged in a lipid bilayer [152-159]. Overall, we can say that lipid bilayers are heterogeneous in a horizontal and vertical direction, while the role of membrane lipids and fatty acids in cell functions is far from being completely understood, e.g. the role of lipid rafts have been intensively studied [160, 161]. Lipid rafts are plasma membrane microdomains that are enriched in cholesterol, glycosphingolipids, and plasmenylethanolamines containing arachidonic acid [162, 163]. They are characterized by a more tightly packed state. Lipid rafts are well-known modulators of the activity of a number immunoreceptors [164] and GPCRs, including cannabinoid receptors [165].

The polar heads of membrane lipids usually consist of a negatively charged phosphate group with bound positive, negative, zwiterionic or uncharged groups. The significance of the polar heads for binding of drugs or function of membrane proteins can be explained on the basis of electrostatic interactions related to the spatial arrangement of the charge in drug or protein surface as well as in polar heads of interacting lipids [166]. It is more difficult to explain the high variability in length and saturation of acyl chains as this heterogeneity is unnecessary for maintaining structure, order and fluidity of lipid bilayer. A likely explanation involves the possibility of accommodating the shape of the acyl chains to the hydrophobic surface of membrane proteins ("hydrophobic matching"), which would enable them to specifically influence the properties of proteins [167]. Phospholipid-binding proteins are an important constituent of signal transduction, molecule transfer and cell metabolism [168]. The hydrocarbon chains of the protein-bound lipids are conformationally more disordered than in fluid bilayer membranes [169].

Saturated and monounsaturated fatty acids can be synthesized in the body *de novo*, but essential polyunsaturated fatty acids are synthesized from food precursors, linoleic acid (18:2) for n-6 group and α-linoleic acid (18:3) for n-3 group of fatty acids. Brain phospholipids are rich in arachidonic acid (20:4, n-6), eicosapentaenoic acid (20:5, n-3), and docosahexaenoic acid (22:6, n-3) bound in the *sn*-2 position of the glycerol core of phospholipids. As mentioned above, arachidonate bound in the position of *sn*-1 phospholipids is rare but sufficient for the formation of anandamide.

Extracellular, membrane and intracellular essential fatty acids and their metabolites can influence a number of events related to signal transduction, gene expression, growth or death of cells, motility and adhesion [108]. It is interesting from the point of view of the psychotropic effects of endogenous cannabinoids (arachidonic acid derivatives) that the role of essential unsaturated fatty acids has been debated for a long time in some biochemical hypotheses of affective disorders, schizophrenia, and neurodegenerative diseases [170-173]. These hypotheses are derived from an assumption that normal neuronal phospholipid metabolism is necessary for normal development and function of the brain. The symptoms of schizophrenia thus may for instance be due to increased speed of elimination of arachidonic and docosahexaenoic acid from neurons [170] Further, the membrane hypotheses of affective disorders assume that the increased ratio of dietary n-6 to n-3 essential fatty acids lead to increased vulnerability to depression [171].

Cholesterol is able to bind anandamide, presumably by interacting with more extended part of the acyl chain, and thus may act as a receptor for anandamide [174]. Furthermore, it was demonstrated that CB_1 receptor and the anandamide transport critically depend on membrane cholesterol content [175]. Accumulating evidence shows that CB_1 receptor binding and signaling, as well as anandamide transport, are under control of lipid rafts. Lipid rafts could control CB_1 signaling not only by modulating the receptor itself, but also by affecting accessory proteins involved in the attenuation of receptor responsiveness through desensitization, internalization and degradation [151]. Unlike CB_1 receptor, no effect of raft perturbation was observed on CB_2 receptor binding and signaling [176]. Since CB_1 and CB_2 receptors recognize the same agonists, trigger the same signaling pathways and are often coexpressed in the same cells, it seems remarkable for their respective functions that they are associated to different membrane domains. The data introduce a new concept, that anandamide-induced signal transduction can be regulated by lipid-lipid interactions on the plasma membrane.

7. STRUCTURE-ACTIVITY RELATIONSHIP OF CANNABINOIDS

The specificity of cannabinoid-protein interactions is related to the multiple covalent bonds of polar and hydrophobic groups of these molecules which require suitable spatial arrangement of protein binding site and cannabinoid molecule. The non-specific binding of cannabinoids to the lipid part of cell membranes, i.e. cannabinoid-lipid interactions are especially determined by hydrophobic effect (during incorporation of cannabinoid molecule into the membrane) and short-range van der Waals forces (during interactions with tails of fatty acids in the hydrophobic core of the bilayer). This is similar to the membrane binding of other lipophilic or amphiphilic psychotropic drugs [177, 178]. Structure-activity relationships (SARs) information about cannabinoids was summarized by Howlett *et al*. [9].

The overall shape of endogenous cannabinoid molecules, as well as the shape of membrane lipid molecules, is determined by conformation of their hydrocarbon chains. At the same time, changes in the spatial arrangement are facilitated by relatively free rotations around simple C-C bonds. The double bonds of unsaturated fatty acids have the *cis* (Z) configuration, which causes a rigid bend of 30° in the hydrocarbon chain. The greater representation of the double bonds leads to very complex molecule shapes. The relationship between structure and activity of anandamide, 2-AG and other cannabinoids has been studied intensively with the goal of determining structural requirements for synthetic agonists and antagonists of the cannabinoid receptors and for the substrates or the inhibitors of the FAAH and the anandamide transporter [179-181].

Great torsional mobility is a significant attribute of the acyl chain of arachidonic acid; it is facilitated by the fact that the *cis* double bonds are separated by one methyl group, which gives these chains greater flexibility in comparison with other unsaturated acyl chains [182]. Therefore, arachidonic acid may occur in many conformations. The four most common are: 1. extended conformation, 2. *U*-shaped conformation, 3. *J*-shaped conformation, and 4. helical conformation. In water, arachidonic acid minimizes exposure of its hydrophobic parts by forming a more compact *U*-shape [183]. Like arachidonic acid, anandamide also occurs in an aqueous environment in many different conformations although mixed extended and *U*-shaped conformations are predominant. As far as 2-AG in water are concerned, *U*-shaped structures also predominate. The extended and the *U*-shaped conformations occur in nonpolar environments also. The angle-iron/extended conformation and hairpin/*U*-shape conformation of anandamide, 2-AG and arachidonate molecules are schematically outlined in Figs. (**5-7**).

It has been confirmed that high affinity binding of anandamide to CB_1 receptors requires the presence of a carbonyl group in the anandamide head group [184]. By contrast, a hydroxyl group is not significant for receptor interaction. The high affinity binding depends on the great flexibility of the anandamide acyl chain. Its analogues with three and more double bonds exhibit high affinity for CB_1. It is assumed that interaction of anandamide with a CB_1 receptor is related to its highly bent conformation. This hypothesis is also supported by comparison of possible anandamide molecule shapes to the Δ^9-THC molecule shape, when good overlaps of molecular volumes are due to the folded conformations of anandamide (especially *U*-shape) [185, 186]. This can explain the fact that molecules as different as endogenous cannabinoids and Δ^9-THC competitively bind to the same receptor. Specific groups which affect activation of cannabinoid receptors have been identified in the Δ^9-THC structure also. These especially involve phenolic hydroxyl, side acyl chain and methyl groups [57]. Sufficient length of saturated tail of acyl chain is a necessary part of the cannabinoid pharmacophore. Similarly it has also been confirmed that the structure of 2-AG is strictly recognized by the cannabinoid receptors and that its structural analogues only exhibit weak agonistic action to CB_1 as well as CB_2 receptors [181].

Structure-activity relationships of cannabinoids and other molecules which influence the cannabinoid system in the brain is being intensively studied with the goal of developing pharmaceutical preparations with the therapeutic effects attributed to the phytocannabinoids [187, 188] without concurrent negative effects on cognitive function. In addition to agonists and antagonists of CB_1 receptors, these involve inhibitors of anandamide membrane transporter and enzymes that catabolize the cannabinoids.

8. ACUTE EFFECTS OF CANNABINOIDS ON THE CNS

A large number of studies have focused on higher brain functions in people intoxicated by cannabinoids [189]. Studies of THC effects on the neurochemistry of experimental animals enable us to understand better the action of the drug on the human brain. The acute reinforcing effects of cannabinoids have been hypothesized to involve release of dopamine in the nucleus accumbens, but also release of endogenous opioid peptides and other transmitters which are independent of the dopamine system [2, 190]. In terms of behavioral effects, THC shows mixed effects of both sedative hypnotics and psychedelics.

The acute effects of psychotropic cannabinoids depend on dose, route of administration, previous experience of the cannabis user and individual susceptibility to psychotropic action of Δ^9-THC, and also on the state of his/her state of mind at the time of taking the drug. With low doses of Δ^9-THC, a combination of stimulating and sedative effects takes place, while sedative effects prevail when higher doses are taken [191]. Low and medium doses of psychotropic cannabinoids (2-10 mg of Δ^9-THC) especially cause qualitative changes in sensory perception, a feeling of comfort, euphoria, relaxation and sedation; however, a worsening of initial bad mood or anxiety may also occur. Medium doses (10-20 mg of Δ^9-THC) may lead to an intensification of emotional responses and reactivity, and to more prominent changes in perception and transient hallucinations. More serious adverse effects (Table **1**) usually occur only with higher doses (>20 mg of Δ^9-THC) but they may also occur with lower doses due to high interindividual variability in response to cannabinoids [192, 193]. From this point of view, dosage of the drug is easier to control when it is smoked than when it is used orally.

Table 1. Adverse Acute Effects and Risks of Cannabinoids [192]

Anxiety and panic
Impaired attention
Impaired short-term memory
Disturbed response time, psychomotor skills and coordination
Increased risk of traffic accidents
Increased risk of psychotic symptoms among vulnerable persons

Acute toxicity of Δ^9-THC is low and death resulting from THC use has not been described [193]. Nevertheless, cardiovascular complications in association with cannabis use have been reported during the past three decades [21, 194]. Cannabinoids may contribute to the cardiovascular collapse associated with myocardial infarction [24]. Elevated levels of endocannabinoids have been related to the extreme hypotension associated with various form of shock as well as to the cardiovascular abnormalities that accompany cirrhosis. It seems that cannabis may be a much more common cause of myocardial infarction than is generally recognized. In contrast, cannabinoids have also been associated with beneficial effects on the cardiovascular system, such as a protective role in atherosclerosis progression and in modulation of the ischaemic-reperfusion injury [24, 195, 196]. Endocannabinoids have been implicated in the protective effects of ischaemic preconditioning through cannabinoid receptor-dependent and -independent mechanisms.

Intoxication with cannabis drugs is usually described as a pleasant experience (laughter, talkativeness, increased sensory perception, increased sexual desire and experience). Still, unpleasant feelings (anxiety, panic, fear) may also take place and the period of comfort can be replaced by a dysphoric phase, and activity can be replaced by a phase of sedation or sleep [2]. Cannabis has a marked negative effect on short-term memory but it seems that the ability to recall previously learned information is not disturbed. Acute effects of cannabis on working memory disappear after 3-4 hours; the degree of damage to memory and other intellectual functions due to regular use of high doses of cannabinoids is still subject to research [57]. Another effect of cannabinoids involves increased appetite. Somatic effects [20] involve hyposalivation with a dry feeling in the mouth and throat, increased heart activity, reddish conjunctiva, reduced tear flow, sometimes orthostatic hypotension with tendency to fall (seldom with a syncope) and increased blood pressure when lying down.

Disturbance of short-term memory, psychomotor functions and tendency to prolong response time after the use of cannabis is similar to that after alcohol intoxication. However, time perception is the opposite. Cannabis makes the internal clock tick faster (a minute seems like several minutes), while alcohol slows it down [57]. Marijuana smokers are rather relaxed and calm, while alcohol can release aggressive and violent behavior. Long-term use of high doses of alcohol usually leads to a permanent damage of intellectual functions, organic brain damage, psychosis and dementia. Similar effects have not been proven for cannabis but it is still unclear to what degree brain development and functions of very young users of this drug are influenced.

Most acute effects associated with cannabis use are reversible. For studies of the changes in brain functions during intoxication with cannabinoids, measurement of regional cerebral blood flow (rCBF) and metabolism (CMR) using magnetic resonance or positron emission tomography has shown that cannabis use is associated with an overall increase in rCBF, which lasts for up to two hours, while significant increases were observed in frontal, insular and anterior regions of cingulate. These changes were greater in the right hemisphere [197]. Increase in rCBF in frontal and temporal cortexes is associated with disturbances in short-term memory. Cannabis induced depersonalization correlates with an increase in rCBF in the right frontal cingulate and in the right frontal region [198]. Cannabis induced changes in time perception are probably associated with changed blood flow in cerebellum [199]. Changes in the total CMR of glucose in brain due to THC varied, but there were always significant changes in the cerebellum [200].

9. CHRONIC EFFECTS OF CANNABINOIDS ON CNS

While acute effects of THC are well-known, knowledge of the influence of chronic cannabis use on cognitive function, neurochemical processes, endocrine and immune systems is not so well-understood [192, 193, 201]. Impaired cognitive functions are more marked among those who started to use cannabis during adolescence [202].

The health risks of chronic marijuana smoking are similar to those of tobacco smoking [203]. Most users seem to smoke cannabis with tobacco. Pulmonary consequences of marijuana smoking may be magnified by the greater deposition of smoke particulates in the lung due to differing manner in which marijuana is smoked. The 1:2.5-5 dose equivalence between cannabis joints and tobacco cigarettes for adverse effects on lung function is of major public health significance [204]. Marijuana smoke can have harmful effects on the heart; but one of its active components may easy inflammation and may slow the progression of coronary artery disease [205]. Physiologic, clinical or epidemiologic evidence, that marijuana smoking lead to chronic obstructive pulmonary disease or respiratory cancer, is limited and inconsistent [206-210].

According to neuropsychological studies, many chronic effects of cannabinoids are deemed likely (Table **2**) and many other are assumed [28, 192, 210, 211]. In addition to the disturbance in short-term memory and attention, they especially involve disturbance in the ability to organize and integrate complex information. Although these disturbances usually do not have a critical impact on smokers´ ability to function relatively normally, a clear impairment of the brain's executive functions is taking place and there is an increased risk of all types of injuries [213].

The hypothesis according to which abuse of cannabis drugs is associated with an increased risk of addiction to other drugs is subject to discussion as the neurobiological basis of this association has not been proven and in fact may be a social phenomenon [57, 214, 215]. Considerable attention is given to the effects of cannabis drugs on the development of adolescents, their ability to pursue further

Table 2. Most Probable Adverse Chronic Effects of Cannabinoids [192]

Chronic bronchitis and histopathological changes
Cannabinoid dependence syndrome
Impairment of attention
Impairment of short-term memory
Disturbance of the ability to organize and integrate complex information
Other side effects are assumed but have not been proven

education and the onset of an "amotivational syndrome" [216]. Chronic cannabis users can be apathetic, lethargic, withdrawn and unmotivated; however, these can rather be symptoms of depression, chronic intoxication and disturbed cognitive functions than symptoms of an amotivational syndrome. On the other hand, it can be deemed proven that long-term use of cannabinoids does not induce a marked disorder in immune functions and does not cause clinically significant genetic changes.

Evidence for structural brain changes in cannabis users is insufficient. Some studies have found no cerebral atrophy or global or regional changes in tissue volumes [217] other studies found grey and white matter density changes; early onset cannabis users were found to have smaller whole brain volumes, lower percent cortical grey matter and larger percent white matter compared to later onset users [218, 219]. Significant reduction of hippocampal and amygdala volumes in long-term very heavy cannabis users was reported [220] and association was found between chronic cannabis use and diminished neuronal and axonal integrity in the dorsolateral prefrontal cortex [221].

There is evidence that heavy users exhibit some cognitive deficits lasting for many days after discontinuing cannabis use [26-28]; however, most of cognitive abilities are unaffected after a few weeks of abstinence [29-31]. Persistence of cannabis-related effects after 28 days of abstinence by the very heavy users of marijuana [27] could be explained by consumption of a significantly greater amount of cannabis by significantly younger users of significantly lower IQ compared to other study [29]. Adolescents appear more adversely affected by heavy use of cannabis than adults. It was summarized that adolescents demonstrate persisting neurocognitive abnormalities related to heavy marijuana use for at least six weeks following discontinuation, particularly in the domains of learning, memory, and working memory [222]. Unfortunately, among youth who have used cannabis repeatedly cannabis use is fairly stable and rates of remission relatively low until age 34 years [223].

Recent studies of cannabis users in the unintoxicated state evidenced that long-term heavy cannabis use is associated with impaired memory function. Deficits have been shown to increase as a function of frequency, duration, dose and age of onset of cannabis use. There is likely to be a wide range of individual differences in the propensity to develop memory dysfunction associated with long-term heavy cannabis use. The evidence suggests impaired encoding, storage, manipulation and retrieval mechanisms in long-term or heavy cannabis users [220].

Although the risk of negative effects of cannabinoids during pregnancy probably is not high, it is preferable to avoid all drugs, including cannabis, during this period. Some studies reported that cannabinoids influence hormone levels, e.g. through action on the hypothalamic-pituitary-adrenal axis [224]. Moreover, the role of endocannabinoid systems in human pregnancy may be crucial, because successful pregnancy implantation and progression seem to require low levels of anandamide [225]. Cannabis does not have a long-term negative impact on global intelligence [226]; however, prenatal cannabis exposure has a significant effect on school-age intellectual development [227], learning, memory and impulsivity [228].

Tolerance develops after chronic cannabinoid use. However, this does not involve tolerance to all effects and does not occur at the same speed and intensity [229]. Well-described mechanisms for tolerance development to GPCR agonists include down-regulation (comprised of both removals of receptors from the cell surface by internalization, and decreased receptor synthesis) and desensitization-uncoupling from effectors (e.g. G proteins). The rate of desensitization of CB_1 was independent of agonist efficacy. So, CB_1 agonist efficacy and rate of desensitization are not necessary related [230]. As far as humans are concerned, only repeated high doses of THC cause tolerance to cardiovascular and psychic effects of cannabinoids. Discontinuation of chronic use of cannabinoids can cause the so-called rebound phenomenon, for instance, an increase in intraocular pressure, loss of appetite etc. Tolerance and withdrawal states, or, more accurately, "withdrawal syndrome", have been proven in experimental animals. However, abrupt interruption of the intake of cannabinoids can cause a withdrawal in some chronic marijuana smokers, involving decreased appetite/weight loss, irritability, anger, aggression, agitation, restlessness, anxiety and sleep disorders. Less frequent symptoms of the cannabis withdrawal syndrome involve tremors, depressive mood, abdominal pain, emotional lability and sweating [231, 232]. These symptoms occur in approximately 16% of heavy marijuana smokers, but they are usually mild. The long elimination half-time of cannabinoids probably contributes to this effect.

10. CANNABINOIDS AND ADDICTION

The class of addictive disorders includes psychoactive substance addiction in which the characteristics that are both necessary and sufficient for identifying a pattern of drug use as drug addiction are fulfilled. Drug addiction is a condition in which a drug that can function both to produce pleasure and to reduce painful affects is employed in a pattern that is characterized by two key features: 1. recurrent failure to control the use one or more drugs, and 2. continuation of drug use despite significant harmful consequences [233]. So, neither tolerance nor withdrawal is necessary or sufficient for a diagnosis of drug addiction. According to the integrative review of Goodman [233] the addictive process can be understood to involve impairments in the three functional systems: motivation-reward, affect regulation, and behavioral inhibition.

Effects of addictive drugs on modulation of the brain reward circuitry are based on the alteration of long-term synaptic plasticity. The reward circuit, also referred to as the

mesolimbic system, is characterized by the interactions of several areas of the brain; ventral tegmental area, nucleus accumbens, prefrontal cortex, basolateral amygdala, and hippocampus are included in drug addiction. In addition to the reward circuit, it is hypothesized that stress mechanisms also play a role in addiction [234]. Converging evidence indicates that the endocannabinoid system is an important constituent of neuronal substrates involved in brain reward processes and emotional responses to stress [24, 235-237].

Several groups of compounds that produce different pharmacological effects can lead to addictive behavior, including cannabinoids, alcohol, nicotine, opioids and psychostimulants. The mesocorticolimbic dopaminergic pathways, the endogenous opioid system, and the brain and pituitary stress system show the crucial role in the addictive processes. However, many other neurotransmitters, neuromodulators and their receptors that underlie long-term associative memories in several forebrain circuits are involved [233, 238]. The common mechanisms involved in the development of the addictive processes have not been yet completely identified; the recent findings support participation of the endocannabinoid system in the common circuitry underlying drug addiction. The CB_1 receptor and its endogenous ligands are understood to reinforce both the motivation and the reward functions of the mesolimbic dopaminergic system in its regulation of eating behavior [239]. Chronic stress was reported to down-regulate CB_1 receptors expression and significantly reduce the content of the 2-AG within the hippocampus [240]. It is suggested that anxiety-related effects of cannabinoids depend on the relative cannabinoid responsiveness of GABAergic and glutamatergic neurotransmission [241]. The primary function of the endocannabinoid system seems to be regulation of chronic stress. Disruption of the endocannabinoid system would be likely to increase the level of chronic stress, which in turn would increase the likelihood of an addictive disorder developing [233].

The endocannabinoid system participates in the primary rewarding effects of cannabinoids, nicotine, alcohol and opioids through 1. the release of endocannabinoids in the ventral tegmental area, 2. involvement of endocannabinoid system in the motivation to seek the drug by a dopamine-independent mechanism, 3. participation in the common mechanisms underlying relapse to drug-seeking behavior, probably by acting on the synaptic plasticity underlying memory processes. The antagonists of CB_1 receptors might represent a new generation of compounds to treat drug addiction [242].

For years, the conventional wisdom held that cannabis was not truly addictive, but this view has been altered due to rapid progress in the understanding of pharmacology and neurochemistry of cannabinoid dependence and abuse [37]. Abstinence from cannabis use by chronic users seldom results in development of pronounced signs of withdrawal, indicative of physical dependence. It is likely due to slow release of THC from fat tissue.

In comparison with opiates, cocaine, alcohol, tobacco or benzodiazepines, susceptibility to cannabis addiction is relatively low [2, 203, 212]. Addiction to cannabinoids is more based on psychological factors than physiological ones (note: abuse of addictive substances with physiological dependence is diagnosed when tolerance or a withdrawal state have been proven). At least some diagnostic guides for dependence syndrome (a cluster of behavioral, cognitive, and physiological phenomena that develop after repeated substance use and that typically include a strong desire to take the drug, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state) are encountered relatively commonly, especially among those who use this drug several times a day. Various studies report the percentage of pure cannabis users who can be classified as dependent ranges from 2 to 10 per cent [243]. Lifetime prevalence of cannabinoid dependence is the third most common diagnosis of addictions, after tobacco and alcohol. At the same time, the risk of an onset of dependence is independent of the time of using the drug, but it increases with quantity and frequency of cannabis use. An increased risk (nearly 22%) of an onset of later cannabinoid dependence was observed among persons with a positive reaction to the use of cannabinoids at an age under 16 [244, 245].

Acute administration of THC provides excitatory input to dopaminergic neurons in the ventral tegmental area through activation of CB_1 receptors. It also increases extracellular levels of dopamine in the nucleus accumbens shell, a brain area involved in the reinforcing and addictive actions of drugs abuse. Interactions between cannabinoid, opioid, and dopaminergic systems are believed to be of primary importance for the expression of rewarding effects of cannabinoids and development of cannabinoid physical dependence [37, 190] In addition to the reinforcing effect of cannabis, its ability to produce a withdrawal state [231, 232] in dependent individuals facilitates dependence. A number of psychotherapies have been found to be effective in treatment of cannabis use disorder; however, there are fewer outpatient treatment studies that have investigated pharmacological agents to treat cannabis-dependent individuals [246].

11. CANNABINOIDS AND MENTAL DISORDERS

The human endogenous cannabinoid system has also been studied from the viewpoint of mental disorders as it interferes with the function of the neurotransmitter systems involved in the pathophysiology of schizophrenia, mood disorders, anxiety and neurodegenerative illnesses [24, 41, 247-251]. The use of cannabinoids leads to cognitive deficits of a similar nature to those seen in schizophrenia [28] and may worsen symptoms of schizophrenia. It is not yet clear to what degree the condition of the cannabinoid system in the brain or intoxication by cannabinoids participates in the onset of other mental disorders, including depression, bipolar or anxiety disorders [252-255]. These studies are also complicated by the fact that the effects on neurochemical processes in the brain induced by administration of psychotropic substances, such as cannabinoids, antidepressants and antipsychotics, are greater than the changes which are related to the mental disorder itself [256, 257]. In addition, we usually are referred to measured biochemical values in peripheral blood, urine or cerebrospinal fluid, while their correlation with brain levels is not always clear. Further, there are large interindividual differences in measurable biochemical parameters even in untreated healthy control persons. We lack studies which monitor drug-naïve persons before administra-

tion of a drug, after acute and long-term administration and subsequent withdrawal.

Some authors believe that there is now enough evidence to inform people that using cannabis could increase their risk of developing a psychotic illness later in life. The evidence that cannabis use leads to affective disorders is less strong than for psychosis [258]. However, data on population rates of both cannabis use and schizophrenia are neither abundant nor convincing, and a substantial increase in schizophrenia has not been seen after apparently substantial increases in population cannabis exposure. So, available evidence does not strongly support an important causal relation between cannabis use and psychosocial harm, but cannot exclude the possibility that such a relation exists [259-261].

Nonetheless, it has been proved clearly that cannabis drugs can induce latent schizophrenic psychosis in susceptible persons, or may deteriorate the disease process in schizophrenic patients [262, 263]. In vulnerable individuals, a psychotic disorder ("toxic psychosis") may occur after using the drug. This usually lasts for a longer time and, for instance, may involve vivid sensory hallucinations, identity disorder, paranoid delusions, touchiness, psychomotor disorders and abnormal emotional states. However, non-clinical positive psychotic experiences are much more common. At the same time, genetic susceptibility, frequency of cannabis use and starting to use the drug during early adolescence are the most important factors [2, 28, 244, 264-267].

There are evidences for functional neural interactions between cannabinoid and dopamine receptor systems [268]. Disturbances in this neural circuitry may be concerned in addiction and schizophrenia. So, cannabinoid hypothesis has been developed as one of the pharmacological etiologies for schizophrenia: enhanced signaling of the cannabinoid system, which is mediated by CB_1 receptors on GABA interneurons in the ventral tegmental area, basolateral amygdala and medial prefrontal cortex, may lead to hyperdopaminergic and hypoglutamatergic state, which may underlie some of the symptoms of schizophrenia [24, 268-270]. The cannabinoid hypothesis for the pathogenesis of schizophrenia is supported by the discovery of a significant association between hebephrenic schizophrenia and polymorphism of the gene for the cannabinoid CB_1 receptor [271]. Further, biochemical analyses have shown that increased cerebrospinal concentrations of endogenous cannabinoids can be found in schizophrenic patients [272], and that frequent use of exogenous cannabinoids lowers anandamide concentrations in cerebrospinal fluid of schizophrenic patients [256]. It can be speculated that CB_1 antagonists may be beneficial against some symptoms of schizophrenia. Δ^9-THC also disturbs mitochondrial function and cellular energetics [273, 274] and this may be related to the unfavorable pulmonary consequences of marijuana smoking as well as to the course of mental disorders [275, 276].

Several lines of evidence suggest that the endocannabinoid system play a role in the regulation of mood, anxiety or addiction, as well as in the pathogenesis and treatment of depression and other stress-related disorders [24, 250, 251]. The overlap between the intracellular functions altered by depression and those affected by CB_1 receptor signaling is notable. Preclinical and clinical data indicate that stress and depression lead to atrophy and loss of neurons in the adult hippocampus; chronic antidepressant treatment up-regulates BDNF and hippocampal neurogenesis. Current efforts are aimed at understanding how CREB and trophic factors are coupled to neurotrophic and neuroprotective effects during long-term antidepressant administration [277, 278]. It is suggested that up-regulation of BDNF might underlie neuroadaptive responses to cannabinoids [277]. Chronic administration of the major drugs of abuse including opiates, cocaine, alcohol, and nicotine decrease adult hippocampal neurogenesis, but chronic treatment with the potent synthetic cannabinoid HU210 promoted neurogenesis in the hippocampal dentate gyrus of adult rats and exerted anxilolytic- and antidepressant-like effects [279]. Cannabinoids appear to be the only illicit drug whose capacity to produce increased hippocampal newborn neurons is positively correlated with its antidepressant effects. Pharmacological modulation of the endocannabinoid system has been proposed as a novel therapeutical strategy for the treatment of stress-related mood disorders such as anxiety and depression [251].

12. POTENTIAL THERAPEUTIC EFFECTS OF CANNABINOIDS

From a clinical point of view, Δ^9-THC, its metabolite THC-COOH, non-psychotropic CBD, various analogues of cannabinoids and newly discovered modulators of the endogenous cannabinoid system exhibit some therapeutic effects. A comprehensive overview on the current state of knowledge of the endocannabinoid system as a target of pharmacotherapy was given by Pacher *et al*. [24].

The favorable effects of cannabinoids on nausea and vomiting (as side effects of anti-tumor therapy), anorexia and cachexia can be regarded as proven. Effects on the spasticity related to spinal cord injuries, movement disorders (Tourette's syndrome, dystonia, Parkinson's disease, tremor, tardive dyskinesis), asthma and glaucoma are also relatively well-confirmed. Effects on allergies, inflammation, infections, epilepsy, addiction and withdrawal syndromes (in addition to benzodiazepines, opiates and alcohol) are less confirmed. In terms of psychiatric syndromes, these involve effects on reactive depression, sleep disorders, anxiety disorders and bipolar disorders. The neuroprotective effects of THC and its effects on autoimmune illnesses, cancer and blood pressure disorders are still being researched [1, 2, 24, 280-285].

The action of cannabinoids on signal transduction is mainly an inhibitory one, which is indicative of their potential role in treatment of illnesses where inhibition of neurotransmitter release is appropriate [286]. Agonists and antagonists of cannabinoid receptors have been developed as well as reuptake inhibitors of endogenous cannabinoids and inhibitors of their intracellular hydrolysis. Accordingly, the molecular targets of medication which interferes with the cannabinoid system involve CB_1 and CB_2 receptors, as well as transport proteins and enzymes which catabolise the endocannabinoids [24] (Table **3**).

No medicines or drugs are completely safe, and hence there is no use in discussing whether cannabinoids are substances whose use has no danger for health. Without doubt, cannabis is a substance abuse with harmful side effects. It is not as dangerous as cocaine, heroin or amphetamines [203] but its seriousness is amplified by the fact that it is the most

common illicit psychotropic drug. Hence, the socioeconomic consequences of its abuse can hardly be compared with those of other addictive substances, apart from alcohol, caffeine and nicotine.

Table 3. Substances which Affect the Cannabinoid System and are Studied for Possible Therapeutic Effects

Non-psychotropic cannabinoids (e.g. cannabidiol)
Non-psychotropic metabolites of Δ^9-THC
Endocannabinoids and their analogues
Cannabinoids and similar substances which do not function *via* CB receptors
Substances which influence transport and metabolism of endocannabinoids
Antagonists of cannabinoid receptors

The possibilities of medical uses of cannabinoids have been discussed for a long time. Clinical tests of substances which influence the CNS cannabinoid system are only just being carried out and the results will determine whether they will be introduced into Western countries. The fact remains that many patients with acquired immunodeficiency syndrome (AIDS), multiple sclerosis, cancer, glaucoma and other disorders smoke marijuana illegally for relief of their distress.

In some countries, medicines with synthetic cannabinoids are available: dronabinol and nabilone. Dronabinol (Marinol®) is a generic name of Δ^9-THC. The pure substance is a light-yellow resin, and it is almost insoluble in water. Therefore, Marinol® is prepared by dissolving dronabinol in sesame oil. Dronabinol is used to treat nausea and vomiting caused by chemotherapy in people who have already taken other medications to treat this type of nausea and vomiting without good results. In the USA, approximately 80% of Marinol® is prescribed to patients with AIDS (for appetite stimulation), 10% to patients undergoing chemotherapy (against nausea and vomiting) and 10% to patients with other indications [57]. Nabilone (Cesamet®) is a keto-cannabinoid; it is the synthetic analogue of THC, and it can only be prescribed in a few countries (Canada, Switzerland and Great Britain). In a pure form, it is a solid crystalline substance and it is used in a solid form. Clinical tests of this substance focus on its use for the treatment of nausea and vomiting during chemotherapy. Sativex®, a plant-derived cannabinoid extract containing both THC and CBD in a 1:1 ratio, is one of the first cannabis-based medicines to undergo conventional clinical development and to be approved as a prescription medicine. It is an oromucosal (mouth) spray that allows flexible dosing. Sativex was approved as a prescription medicine in Canada in 2005 and is currently under regulatory review in the Europe [287]. The hypothesis that the combination of THC and CBD increases clinical efficacy while reducing adverse events was supported [16].

The CB_1 receptor antagonist rimonabant, also known as Acomplia, has been approved for the treatment of cardiometabolic risk factors associated with obesity. It is still under study for other disorders that have a prominent craving component. It has been proposed that rimonabant may have additional potential applications apart from reducing body weight [195].

Cannabis-based medicine may represent a useful new agent for treatment of the symptomatic relief of spasticity associated with multiple sclerosis and result in some benefit in secondary outcome measures, assessing mobility and patients' perception of the effect of spasticity [288, 289]. Modulation of the endocannabinoid system by Sativex [287, 290-292] or by dronabinol [293] was proved to be effective in the treatment of central neuropathic pain in patients with multiple sclerosis. Sativex was effective, with no evidence of tolerance, in patients who completed approximately 2 years of treatment. Sativex successfully treats neuropathic pain of peripheral origin also [294]. Experiences to date with Sativex demonstrate marked improvement in subjective sleep parameters in patients with a wide variety of pain condition [295]. The most frequent adverse effects of cannabis and THC in clinical studies comprise effects on psyche and cognition, nausea and dry mouth [193]. The most common side effects of Sativex were dizziness and nausea which were deemed to be of mild to moderate severity [193, 291, 292]. Anyway, adverse side effect profile of cannabinoids has generally been mild compared with other drugs used for pain and spasticity.

13. CONCLUSIONS

Cannabinoids are a subject of intense research: both the psychotropic constituent of cannabis and the role of the endocannabinoid system in humans and its relation to various brain disorders including neurodegenerative ones. While acute effects of phytocannabinoids are relatively well-known, insufficient information is available on the effects of the chronic abuse of cannabis drugs. In addition to euphoria and elation, marijuana smoking can also cause anxiety, short-term memory and attention deficits and have many other cognitive, affective and psychomotor effects. Generally, cannabis is a drug of moderate toxicity, particularly when used by adults [193]. It is not yet clear to what degree it disturbs brain development and functions in very young cannabis users, but it has been proven that this group of users has an increased risk of cannabinoid dependence. In vulnerable people, cannabis drugs may induce latent schizophrenic psychosis or may unfavorably influence its course. Heavy marijuana smokers may show increased depressive symptoms in comparison with others. Only further longitudinal studies on psychotic symptoms in society will make it possible to understand the relationship between cannabis use and psychosis, and determine whether there is a critical period in brain development when cannabinoids are especially detrimental.

Specific effects of cannabinoids are due to activation of the cannabinoid system in the brain and processes associated with this. Primary biochemical effects of substances which influence the cannabinoid system consist in agonistic or antagonistic action on cannabinoid receptors, inhibition of enzymes which participate in cannabinoid catabolism, or in influencing the transmembrane transport of cannabinoids. Acute effects of cannabinoids are probably related to activation of presynaptic cannabinoid receptors and inhibition of release of a number of neurotransmitters in the brain. In chronic cannabis users, it is possible to assume adaptive

changes in their cannabinoid system and related neurotransmitter systems; the changes for instance involve regulation of density and sensitivity of membrane receptors and activity of specific neurotransmitter transporters. The modulation of the neuron plasticity induced by long-term activation of postsynaptic and presynaptic CB_1 receptors may be involved in the cannabinoid-induced adaptive changes of neurotransmission which result in adverse or therapeutic effects of cannabinoids.

Endogenous agonists of cannabinoid receptors in brain especially involve anandamide and 2-AG. They are derived from arachidonic acid, one of the most important unsaturated fatty acids in the brain. Variability of the shape of the arachidonic acid, and therefore also the anandamide and 2-AG acyl chain, is necessary for activation of a cannabinoid receptor and it makes it possible for molecules of endogenous receptors to adopt a shape similar to that of much more rigid tetrahydrocannabinols. This can explain the fact that endocannabinoids and Δ^9-THC compete for the same binding site on CB receptor.

Owing to their lipophilicity, the cannabinoids accumulate in the lipid part of cell membranes and they may occupy binding sites localized on hydrophobic portions of integral membrane proteins. This facilitates influence of the function of various membrane proteins which participate in signal transduction. The function of the lipid part of cell membranes and the role of essential fatty acids in this regard, may also be affected. Therefore, the spectrum of possible changes in neurotransmitter systems after the long-term use of cannabinoids is very wide and little known. Understanding them is associated with progresses in learning about normal cellular functions, especially in the field of the effects of the endogenous cannabinoids.

The possible medical uses of cannabinoids have been long debated. Research involves synthesis of cannabinoid analogues with therapeutic effects and none of the psychotropic properties of Δ^9-THC. Understanding the relationship between the structure and activity of cannabinoids may assist the search for substances with therapeutic effects in cases of illness, including disorders induced by clinical endocannabinoid deficiency [296]. In most western countries, cannabis remains prohibited even for medicinal purposes. It seems that the prohibition of medical cannabis tends to affect much more those adult patients with severe diseases that respond positively to cannabis treatment than trafficking and use of cannabis by adolescents [193]. Social background, emotional, and other psychosocial factors appear to be more reliable predictors of cannabis use than the availability of the drug or its legal status. It is substantial that the medical use of cannabinoids appears to offer some persons benefits for a wide range of indications.

ACKNOWLEDGEMENTS

This research was supported by grant No NR8408-3/2005 given by the Internal Grant Agency of the Ministry of Health and by MSM0021620849 grant given by the Ministry of Education, Youth and Sports of the Czech Republic.

Key Learning Objectives:

1. To summarize the major findings related to acute and chronic effects and mechanisms of action of cannabinoids (plant, endogenous, and synthetic).
2. To introduce to the role of membrane lipid bilayer structure and composition in the cannabinoid system and to highlight the structure-activity relationships of cannabinoids.
3. To examine the role of endocannabinoid system in addiction and mental disorders.
4. To summarize potential therapeutic effects of cannabinoids.

Future Research Questions:

1. What are the neurochemical processes that lead to changes in the cognitive, affective and psychomotor function of cannabis users? Verification or refutation of the hypothesis that long-term heavy cannabis use causes irreversible cognitive deficits.
2. What is the role of non-CB_1, non-CB_2 brain receptors in effects of cannabinoids?
3. How are endocannabinoids transported back to cells?
4. Are changes in the neuron plasticity produced by long-term activation of cannabinoid receptors?
5. What is the function of lipid part of cell membranes and essential fatty acids in the activity of endocannabinoid system?
6. Is there a relationship between cannabis use and psychosis or mood disorders? Further longitudinal studies are needed.

ABBREVIATIONS

AC	=	Adenylate cyclase
ACEA	=	Arachidonoyl-2'-chloroethylamide
ACPA	=	Arachidonoylcyclopropylamide
AEA	=	Anandamide, *N*-arachidonoylethanolamide
2-AG	=	*sn*-2-Arachidonoylglycerol
AIDS	=	Acquired immunodeficiency syndrome
AM630	=	6-Iodo-2-methyl-1-[2-(4-morpholinyl) ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone; 6-iodopravadoline
AT	=	Anandamide transport
BDNF	=	Brain-derived neurotrophic factor
cAMP	=	Cyclic adenosine monophosphate
CB_1, CB_2	=	Type-1 and type-2 cannabinoid receptors
CBD	=	Cannabidiol
CMR	=	Cerebral metabolic rate
CNS	=	Central nervous system
COX-2	=	Cyclooxygenase-2
CP47497	=	5-(1,1-Dimethylheptyl)-2-(3-hydroxycyclohexyl)-phenol
CP55244	=	(–)-*Cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexan-1-ol

CP 55,940 = (1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol

CREB = cAMP response element-binding protein

DAG = Diacylglycerol

DAGL = Diacylglycerol lipase

FAAH = Fatty acid amide hydrolase

G protein = Guanine nucleotide-binding protein

GABA = γ-Aminobutyric acid

GPCR = G Protein-coupled receptor

GIRK = Inwardly rectifying K^+-channel

HU-210 = (6*aR*,10*aR*)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6*a*,7,10,10*a*-tetrahydrobenzo[*c*]chromen-1-ol

HU-308 = 4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}-methanol

IP_3 = Inositol-1,4,5-triphosphate

JWH-051 = 3-(1‘,1‘-Dimethylheptyl)-1-deoxy-11-hydroxy-Δ^8-tetrahydrocannabinol

JWH-133 = (6*aR*,10*aR*)-6,6,9-trimethyl-3-(2-methylpentan-2-yl)-6*a*,7,10,10*a*-tetrahydrobenzo[*c*]chromene

K_i = Receptor affinity

L-759633 = (6*aR*,10*aR*)-3-(1,1-Dimethylheptyl)-1-methoxy-6,6,9-trimethyl-6*a*,7,10,10*a*-tetrahydro-6*H*-benzo[*c*]chromene

LOX = Lipoxygenase

MAGL = Monoacylglycerol lipase

MAPK = Mitogen-activated protein kinase

NADA = *N*-arachidonoyldopamine

NAE = *N*-acyl ethanolamine

NAPE = *N*-arachidonoyl phosphatidylethanolamine

PIP_2 = Phosphatidylinositol-4,5-biphosphate

PKA = Type A protein kinase

PLA = Type A phospholipase

PLC = Type C phospholipase

PLD = Type D phospholipase

rCBF = Regional cerebral blood flow

SAR = Structure-activity relationship

SR141716A = *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; rimonabant

SR144528 = *N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide

SR147778 = 5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4-ethyl-*N*-(1-piperidinyl)-1*H*-pyrazole-3-carboxamide

Δ^9-THC, THC = (−)-(6*aR*,10*aR*)-6,6,9-trimethyl-3-pentyl-6*a*,7,8,10*a*-tetrahydro-6*H*-benzo[*c*]chromen-1-ol; Δ^9-tetrahydrocannabinol; dronabinol

11-OH-THC = 11-Hydroxy-Δ^9-tetrahydrocannabinol

THC-COOH = 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid

THC-COOHglu = 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-glucuronide

TRPV1 = vanilloid/capsaicin receptor

WIN 55,212-2 = (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone

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Received: January 16, 2008 Revised: March 12, 2008 Accepted: June 12, 2008