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Cannabinoids and Cytochrome P450 Interactions

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Abstract: ***Objective:*** This review consists of three parts, representing three different possibilities of interactions between cannabinoid receptor ligands of both exogenous and endogenous origin and cytochrome P450 enzymes (CYPs). The first part deals with cannabinoids as CYP substrates, the second summarizes current knowledge on the influence of various cannabinoids on the metabolic activity of CYP, and the third outline a possible involvement of the endocannabinoid system and cannabinoid ligands in the regulation of CYP liver activity.

Methods: We performed a structured search of bibliographic and drug databases for peer-reviewed literature using focused review questions.

Results: Biotransformation via a hydrolytic pathway is the major route of endocannabinoid metabolism and the deactivation of substrates is characteristic, in contrast to the minor oxidative pathway via CYP involved in the bioactivation reactions. Phytocannabinoids are extensively metabolized by CYPs. The enzymes CYP2C9, CYP2C19, and CYP3A4 catalyze most of their hydroxylations. Similarly, CYP represents a major metabolic pathway for both synthetic cannabinoids used therapeutically and drugs that are abused. In vitro experiments document the mostly CYP inhibitory activity of the major phytocannabinoids, with cannabidiol as the most potent inhibitor of many CYPs. The drug-drug interactions between cannabinoids and various drugs at the CYP level are reported, but their clinical relevance remains unclear. The direct activation/inhibition of nuclear receptors in the liver cells by cannabinoids may result in a change of CYP expression and activity. Finally, we hypothesize the interplay of central cannabinoid receptors with numerous nervous systems, resulting in a hormone-mediated signal towards nuclear receptors in hepatocytes.

Keywords: Cannabinoids, cytochrome P450, endocannabinoid system, interaction, metabolism, regulation.

1. INTRODUCTION

Cytochrome P450 (CYP) enzymes are haem-containing monooxygenases (EC 1.14.14.1) bound to the membranes of the endoplasmic reticulum or mitochondria in the liver, intestine, kidney, lung, brain, skin, and heart, with the highest level of expression in the liver and intestine [1, 2]. CYPs are functionally coupled with cytochrome P450 reductase, which enables the transfer of electrons from NADPH, the reduced form of NADP (nicotinamide adenine dinucleotide phosphate), to CYP. Microsomal enzymes from subfamilies CYP3A, CYP2C, CYP2D, CYP1A, and CYP2B play a pivotal role in the metabolism of xenobiotics [2]. Variability in the drug plasma levels may diverge depending on different factors, and according to some authors may reach up to 40-fold differences [3]. The most important factors influencing drug plasma levels include the activities of the CYPs with their genetic polymorphisms, epigenetic changes such as DNA methylation and histone deacetylation, together with exogenous factors. These factors substantially influencing CYP metabolic activity are the major source of variability in the pharmacokinetics of drugs and thus in drug responses [3]. CYPs are therefore of particular relevance in clinical pharmacokinetics. On the other hand, the importance of CYP in the metabolism of endogenous substances is also crucial. CYPs are involved in the metabolism of steroid hormones, cholesterol, vitamin D, bile acids and eicosanoids [1], and also most endocannabinoids [4].

Cannabinoids are a group of substances originally isolated from the cannabis plant (*Cannabis sativa*). Today over 100 different molecules with similar structure, most of them with a C21 terpenophenolic moiety, have been isolated and described [5, 6]. They are known to have a wide range of pharmacologic effects [7, 8], for which the hemp plant has been used for over 6000 years in herbal medicine and as a recreational drug.

The first cannabinoid isolated from the cannabis oil was cannabinol (CBN) in 1898 [9, 10], followed by cannabidiol (CBD) in 1940 [11]. Nevertheless, the major psychoactive compound of cannabis remained unknown until 1964, when Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated in its pure form [12, 13] and its structure was described [14]. The second breakthrough in cannabinoid research was the finding that Δ^9-THC elicits its activity by binding to specific receptors. The first two G-protein coupled receptors (GPCRs) to be discovered, which when activated inhibit adenylyl cyclase, were called the CB1 and CB2 receptors. The CB1 receptor was identified in the brain in 1988 [15], and the CB2 receptor in immune cells in 1993 [16]. These were the first pieces of direct evidence for the possible existence of the endocannabinoid system [17]. The cloning of both of these receptors [16, 18] opened the door to the identification of their endogenous ligands (endocannabinoids), and to the description of their distribution and transduction signal pathways. Anandamide (N-arachidonoylethanolamine) [19] and 2-arachidonoylglycerol (2-AG) [20, 21] are among the first detected and most studied endocannabinoids so far. More recent studies indicate that endocannabinoids, besides the cannabinoid receptor, can also activate multiple receptor targets, including nuclear peroxisome proliferator-activated receptors (PPARs) [22, 23], the transient receptor potential vanilloid type 1 receptor (TRPV1) [24, 25], and orphan G protein-coupled receptors, such as GPR55, GPR119, and GPR18 [26-29]. Other works indicate that cannabinoids have the ability to modulate the activity of additional receptors and their signal transduction pathways, for example

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opioid, serotonin, NMDA, and nicotinic acetylcholine receptors [29, 30].

Nowadays, the terminology concerning cannabinoids is not unified. Some authors describe cannabinoids as ligands of cannabinoid CB1 or CB2 receptors of herbal (phytocannabinoids), endogenous (endocannabinoids) or xenogenic origin (synthetic cannabinoids). Some others differentiate between a) true cannabinoids with the structure derived from endogenous arachidonic acid or natural herbal cannabis-derived compounds, b) synthetic cannabinoid-like compounds of different structures with either direct or indirect cannabimimetic effects, or compounds inhibiting the cannabinoid receptor activities. Moreover, some authors consider endogenous molecules with a similar structure, but without the ability to bind to CB receptors to also be cannabinoids. These endocannabinoid-like compounds can interfere with the activity of true endocannabinoids, as they are in several cases synthetized and biotransformed via the same pathways [31]. For the above reasons and to maintain the clarity the authors of this review decided to use the name cannabinoids for all of the substances described. An overview of endocannabinoids and endocannabinoid-like substances as well as the most common phytocannabinoids, synthetic ligands of cannabinoid receptors used in preclinical studies, and cannabinoid derived drugs is shown in Table **1**.

The aim of this work is to provide a comprehensive review of the interactions between CYPs and the endocannabinoid system and its ligands. Here, we describe the role of CYP in the metabolism of cannabinoids and vice versa the role of cannabinoids in the regulation of CYP activity.

2. CANNABINOIDS AS SUBSTRATES OF CYTOCHROME P450 MONOOXYGENASES

The endogenous and exogenous cannabinoids are substrates of various CYPs. Due to the possibility of interaction between endocannabinoids, phytocannabinoids, or synthetic cannabinoids and other drugs at the CYP site, there is a risk of treatment failure or drug toxicity. It is therefore important to identify possible sites of such interactions for the successful prevention of pharmacokinetic drug-drug interactions.

2.1. Endocannabinoids and Endocannabinoid-Like Compounds

Numerous amides of fatty acids, notably amides of arachidonic acid, its derivatives, and their metabolites, are potent ligands of cannabinoid receptors. To date, anandamide (AEA), 2-AG and its isomer 1-arachidonoylglycerol, oleamide (oleic acid amide), virodhamine (O-arachidonoylethanolamine), di-homo-γ-linolenoyl-ethanolamide, N-arachidonoyldopamine, noladin ether (2-arachidonylglyceryl ether), and N-arachidonoylserine were identified and proved to be endogenous ligands of at least some cannabinoid receptors. Other endogenous N-acylethanolamines, N-acylethanolamides, and N-acyl-aminoacids such as palmitoylethanolamide, N-arachidonoyltaurine, N-arachidonoylglycine [32-35], N-docosatetraenoyl-ethanolamine, N-docosahexaenoylethanolamine, or N-eico-sapentaenoylethanolamine were found in mammalian tissues over the last decade and exhibit varying affinity to cannabinoid receptors CB1 and CB2. It is also possible that they potentiate the effects of „classical" endocannabinoids such as anandamide and 2-AG independently of binding to CB receptors. Therefore, they are sometimes called „endocannabinoid-like compounds" [4, 35-37].

The metabolism of AEA and 2-AG, being the first investigated and most studied endocannabinoids, was recently reviewed by Snider *et al*. [38] and Zelasko *et al*. [4].

The biological effects of most endocannabinoids are terminated by transport to the cells and enzymatic inactivation. It was hypothesized that the transport of endocannabinoids to the cells may also regulate their biological effects. Mechanisms such as simple diffusion, facilitated diffusion or endocytosis are thought to uptake AEA to the cells [38]. A major degradation pathway is catalyzed by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) [4, 38]. The oxidative degradation of endocannabinoids is only a minor pathway and involves the enzymes cyclooxygenase-2 (COX-2), 12- and 15-lipoxygenase (12-LOX, 15-LOX, respectively), and CYPs [38-41]. Since some of the metabolites of endocannabinoids originating via CYP enzymes are active ligands of CB receptors, the role of this oxidative pathway remains unclear [4, 35, 37, 38]. Due to the focus of this review, CYP-mediated pathways are described in detail.

Table 1. Overview of cannabinoids.

Endocannabinoids	Endocannabinoid-like compounds	Phytocannabinoids	Synthetic cannabinoids
anandamide	palmitoylethanolamide	Δ^9-tetrahydrocannabinol	dronabinol[a]
2-arachidonoylglycerol	N-docosatetraenoylethanolamine	Δ^8-tetrahydrocannabinol	nabilone[a]
noladin ether	di-homo-γ-linolenoyl-ethanolamide	cannabidiol	rimonabant
virodhamine	2-oleoylglycerol	cannabinol	methanandamide
arachidonoyldopamine	N-oleoylethanolamine	cannabigerol	JWH-0133
N-arachidonoylserine	N-eicosapentaenoylethanolamine	cannabichromen	AM-251
homo-γ-linolenoylethanolamide	N-docosahexaenoylethanolamine	cannabivarin	ACEA
			ACPA
7,10,13,16-docosatetraenoylethanolamide	oleamide	cannabielsoin	WIN 55,212-2
	N-arachidonoylglycine	cannabitriol	CP 55,940
	1- arachidonoylglycerol		HU-210
	N-arachidonoyltaurine		

[a] synthetic analogues of Δ^9-THC

2.1.1. Arachidonic Acid

Since the CYP-mediated metabolic pathways of endocannabinoids are closely similar to the metabolism of arachidonic acid (AA), this CYP-mediated metabolism of AA is reviewed briefly so as to elucidate theoretical possibilities of the oxidations at the "fatty acid" site of endocannabinoid molecules.

CYPs are known to metabolize arachidonic acid by epoxidation, ω/ω-1 hydroxylation, bis allylic oxidations, and hydroxylation to conjugated dienols (Fig. **1**) [42-44]. As a result, a wide variety of metabolites with biological activities are produced.

Arachidonic acid has four double bonds and epoxidation may occur on any of them. The products of epoxidation, epoxyeicosatrienoic acids (EET), may be further hydrolyzed to dihydroxyeicosatrienoic acids (diHETE). EET are produced by several hepatic and extrahepatic CYPs - CYP2C8, CYP2C9, CYP1A2, and CYP2B6, with the latter playing only a minor role (Table **2**). The ω/ω-1 hydroxylations of arachidonic acid to hydroxyeicosatrienoic acid (HETE) are catalyzed by the CYP4A, CYP2E1, and CYP4F families (Table **3**). Finally, bis-allylic oxidations and hydroxylations with double bond migration are catalyzed by CYP families 1A, 3A, 2C, and 4F (Table **4**).

2.1.2. Anandamide

Anandamide, the first known endocannabinoid, was isolated from the porcine brain by L. O. Hanuš and W. A. Devane from the team of prof. R. Mechoulam at Hebrew University, Jerusalem in 1992 [19]. AEA is hydrolyzed by the membrane-bound enzyme FAAH, with the highest level of expression in the liver. This degradative pathway is the most important in the regulation of AEA cellular and tissue concentrations. FAAH hydrolyses AEA towards arachidonic acid and ethanolamine. Thus the inhibition of FAAH may become a useful alternative in cannabinergic treatment options [38]. COX-2, an enzyme expressed in an inducible manner in inflammation, converts anandamide to several prostaglandin ethanolamides [46, 47]. Oxidation of the aliphatic chain by 12-LOX and 15-LOX yields 12- and 15-hydroxyanandamide. 12-hydroxyanandamide in particular may play a significant role in the modulation of neuronal functions via its influence on neurotransmitter levels [48].

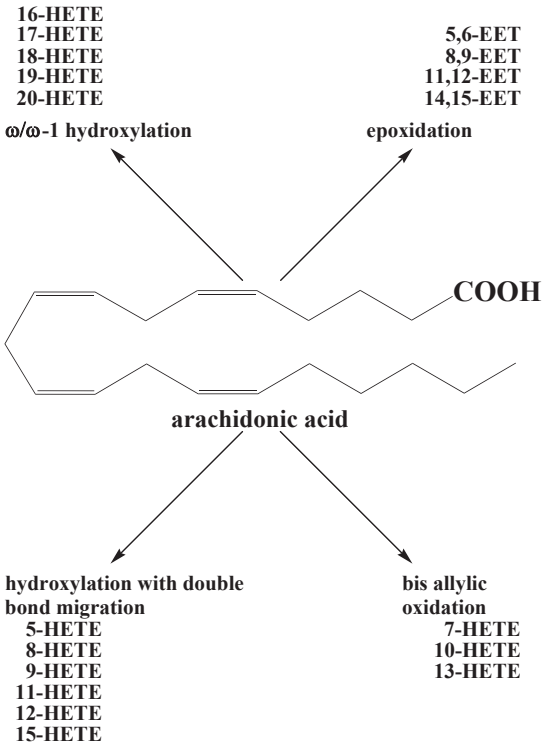

Fig. (1). CYP-mediated metabolism of AA [42-44].

CYPs involved in the degradation of AEA belong to the CYP3A and CYP4F families. The biodegradation of anandamide by CYPs was reported for the first time by Bornheim *et al*. in 1995 [49], who described its conversion by mouse liver microsomal fraction to approximately 20 products, whose structures were not identified. Furthermore, pretreatment with common CYP inducers such

Table 2. Epoxidation of AA catalyzed by cytochrome P450 enzymes [42-44].

CYP	Product	Tissue
2B6	14,15-EET, 11,12-EET	liver
2C8	14,15-EET, 11,12- EET, 8,9-EET	liver, lung, vascular endothelium
2C9	14,15- EET, 11,12- EET, 8,9-EET	liver, lung, vascular endothelium
2C19	14,15- EET, 8,9-EET	liver
2J2	5,6- EET, 8,9- EET, 11,12- EET, 14,15-EET	kidney, GIT, pancreas
1A2	8,9- EET, 11,12-EET	liver, lung

Table 3. ω/ω-1 hydroxylations of AA catalyzed by cytochrome P450 enzymes [44, 45].

CYP	Product	Tissue
4A11	19-, 20-HETE	liver, kidney
4F2	20-HETE	liver, kidney
4F3	hydroxy-LTB_4	polymorphonuclears
4F11	?	liver, kidney
4F12	18-HETE	liver, kidney
2E1	19(*S*)-HETE, 19(*R*)-HETE, 18(*R*)- HETE	

Table 4. Bis-allylic oxidations and hydroxylations of AA with double bond migration which are catalyzed by cytochrome P450 enzymes [42].

CYP	Product	Tissue
1A2	7-, 10-, 13-HETE	liver
3A4	7-, 10-, 13-HETE	liver
2C8	11-, 13-, 15-HETE	liver
2C9	12-, 13-HETE	liver
2C19	13-, 19-HETE	liver
4F8	13-HETE	liver, ovary/testes
4F12	18-HETE	liver, kidney, GIT

as dexamethasone increased the formation of metabolites 5-15 fold, and pre-treatment with a CYP3A antibody diminished the production of anandamide metabolites. Bornheim *et al*. also suggested that the CYP3A, CYP2B, and CYP1A subfamilies are involved in the metabolism of AEA in mouse liver microsomes. Similarly, Costa *et al*. suggested that CYP3A and CYP2B subfamilies are involved in the metabolism of anandamide in rat liver microsomes [50].

To the best of our knowledge, Snider *et al*. was the first to investigate the biotransformation sites of AEA by human liver and kidney microsomal CYPs, and identified the metabolites [51]. The biotransformation routes are fundamentally similar to those of AA. Anandamide may be epoxygenated by several CYPs at positions 5-6, 8-9, 11-12, and 14-15 to form four epoxyeicosatrienoic acid ethanolamides (EET-EAs). At least in some of them, this oxidative pathway is more bioactivation than degradation, since 5,6-EET-EA seems to be a more stable CB ligand than AEA itself [38]. Nevertheless, all EET-EAs may be further hydroxylated in the ω positions (again, similarly to AA metabolism) predominantly by CYP2D6 and thus 20-hydroxy-epoxyeicosatrienyl ethanolamides (HEET-EAs) are produced [51]. With 5,6-EET-EA and 14,15-EET-EA, hydroxylations at positions 16, 17, 18, and 19 were also described. EET-EA may be hydrolyzed by epoxyhydrolase to form dihydroxylated EET-EA [52].

Besides these reactions, ω- and ω-1-hydroxylations of AEA were also described [4, 38, 51, 53]. Details on the oxidative metabolism of AEA are shown in Figure **2**.

2.1.3. 2-arachidonoylglycerol

The main metabolic degradation of 2-AG is catalyzed by MAGL, FAAH, and α,β-hydrolase domains (ABHD) 6 and 12 [4, 38, 54]. The structure of 2-AG suggests that they are subject to the same oxidative metabolism as AA and AEA, which would lead to four regioisomeric 2-epoxyeicosatrienylglycerol derivatives (EET-G) (Fig. **3**). In contrast to this assumption, only 2 EET-G were identified to date – 2-(11,12-epoxyeicosatrienyl)-glycerol and 2-(14, 15-epoxyeicosatrienyl) glycerol, which are produced by CYP2J2 in rat kidney and spleen [55] and in bovine and porcine myocardium [56, 57]. These metabolites demonstrate regulatory effects on blood pressure, as was shown in the study of Awumey *et al*. [58].

As well as in EET-EA, the CYP-mediated epoxygenation of 2-AG to EET-G seems to be a kind of bioactivation, since these metabolites exhibit a tighter binding to CB receptors than 2-AG [56]. EET-G may be oxidatively decomposed by CYP2J2 to AA and glycerol.

2.1.4. N-arachidonoyldopamine

N-arachidonoyldopamine (NADA) is another endocannabinoid known to exert significant biological activity, e.g. in the immune system and pain perception [59, 60]. Besides hydrolysis to AA and dopamine by FAAH [35], NADA may be hydroxylated by rat microsomal protein in the presence of NADPH in the ω and ω-1 positions to form 19- and 20-hydroxyeicosatetraenyl dopamine (19-HETE-DA and 20-HETE-DA) [60] (Fig. **4**).

The question of epoxygenase reactions analogous to the CYP-mediated metabolism of AA and anandamide remains to be further elucidated.

2.1.5. Other Endocannabinoids and Cannabinoid-Like Compounds

The metabolic fate of the other endocannabinoids and cannabinoid-like compounds, such as virodhamine, oleamide, N-arachidonoylglycine, N-arachidonoylserine, or N-arachidonoyltaurine is not well understood, but hydrolysis with esterases or amide hydrolases is likely. On the other hand, hydrolysis of the ether group (e.g. noladin ether) by these enzymes is not likely, in contrast to oxidative metabolism, which may be an alternative degradative pathway for ethers, but there is still no direct evidence for this.

2.1.6. Concluding Remarks Concerning Endocannabinoid Metabolism

The metabolism of endocannabinoids via the hydrolytic pathway (namely FAAH) usually produces inactive metabolites, in terms of their affinity to bind to CB receptors. On the other hand, the products of the oxidative pathway may be both metabolites with a lower affinity to CB receptors (20-HETE-EA and 14,15-EET-EA) and products with a higher affinity to the CB (or PPAR) receptor than the parent compound, as shown with some 2-11,12-EET-Gs and 2-14,15-EET-Gs [38]. Moreover, a molecule with higher stability (5,6-EET-EA) can be produced. From this point of view, the inhibition of endocannabinoid degradation may be a valuable pharmacological target, and has been shown to produce anxiolytic-like and antidepressant-like effects in animal models [62]. Despite promising results from animal studies, there are no reliable data on efficacy from clinical studies. The clinical trials were focused mostly on safety; in general, the inhibitors were well tolerated and lacked typical "cannabinoid-like effects" [62]. There has also been a reported lack of efficacy in a clinical trial of an FAAH inhibitor in the treatment of osteoarthritic pain [63]. Modulation of the oxidative metabolic pathway was not studied in terms of a possible therapeutic approach; modulating the oxidative pathway would be problematic due to the involvement of CYPs (CYP2D6, CYP2C8, CYP3A) in the metabolism of other endogenous substances and possibly also co-administered drugs.

2.2. The Metabolism of Phytocannabinoids *via* Cytochrome P450 Monooxygenases

The term phytocannabinoids covers naturally occurring phytochemicals from *Cannabis sativa*, *Cannabis indica,* or *Cannabis*

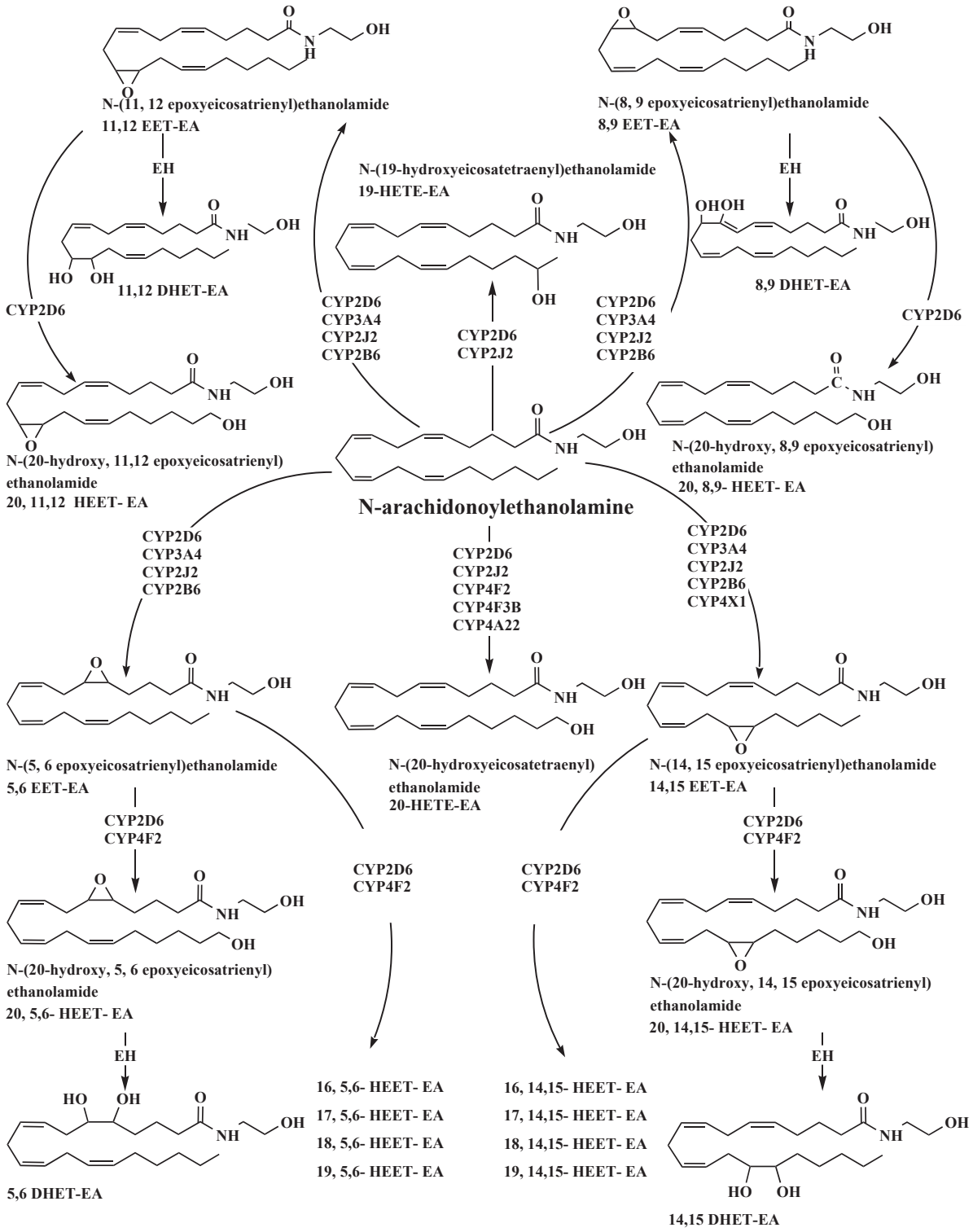

Fig. (2). CYP-mediated metabolism of anandamide [4, 38, 61].

ruderalis which are able to interact with cannabinoid receptors [64, 65]. Nearly 500 chemical entities were identified in *Cannabis* herbage, of which about 70 are phytocannabinoids. These compounds are present in the highest amounts in the viscous resin produced by the glandules of female cannabis inflorescence [64, 66]. As a result, several chemical classes of phytocannabinoids were defined by ElSohly *et al*. [66]: 1) cannabigerol type, 2) cannabichromene type, 3) cannabidiol type, 4) (-)-Δ^9-trans-tetrahydrocannabinol type, 5) (-)-Δ^8-trans-tetrahydrocanna-binol type, 6) cannabicyclol type, 7) cannabielsoin type, 8) cannabinol type, 9) cannabinodiol type, 10) cannabitriol type, and 11) miscellaneous type. In terms of this classification, the (-)-Δ^9-trans-tetrahydrocannabinol type, cannabinol type, and cannabidiol type are the most abundant and best known and studied. Out of 70 known phytocannabinoids, only Δ^9-THC, CBN, and CBD are reviewed in terms of oxidative metabolism by CYPs. No data were found for the other phytocannabinoids.

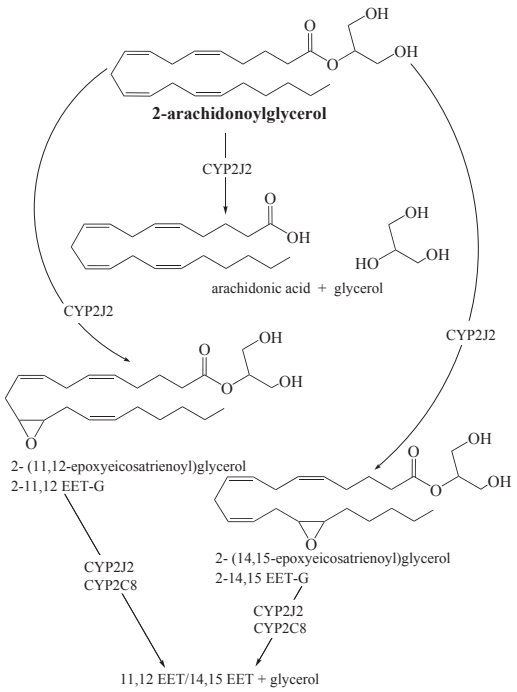

Fig. (3). CYP-mediated metabolism of 2-arachidonoylglycerol [4, 38].

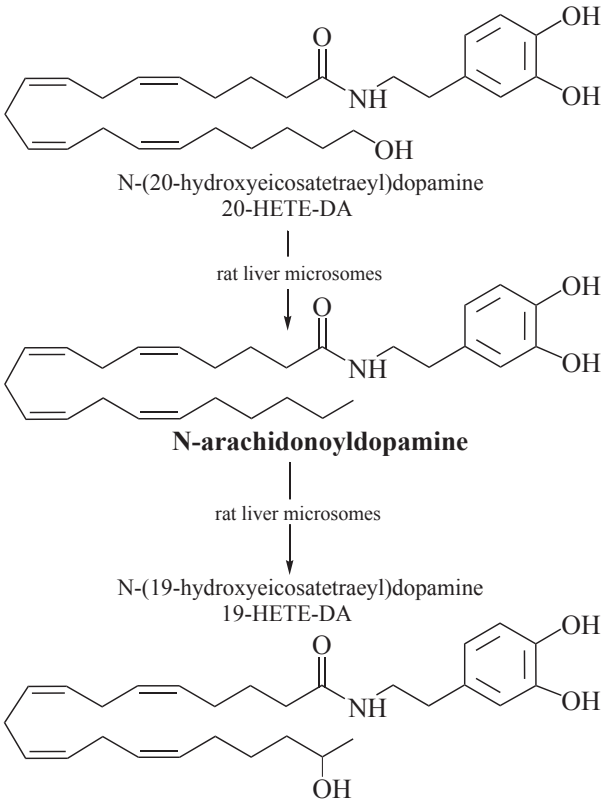

Fig. (4). CYP-mediated metabolism of N-arachidonoyldopamine [60].

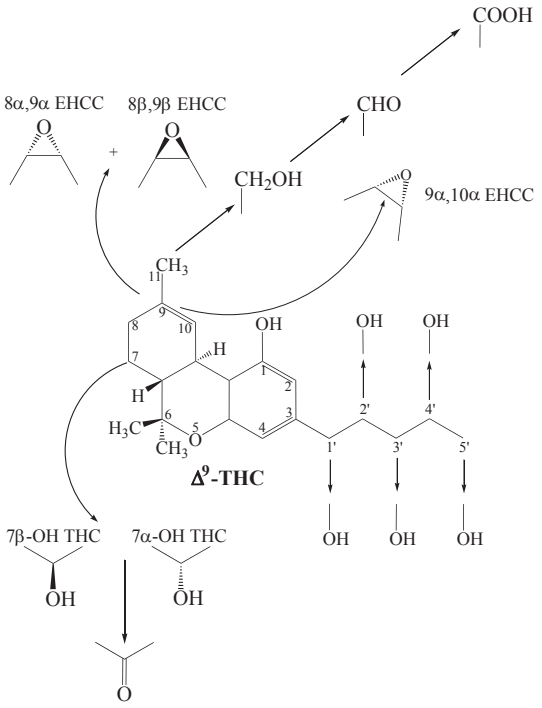

Fig. (5). Structure and CYP-mediated oxidative metabolism of Δ^9-THC [69].

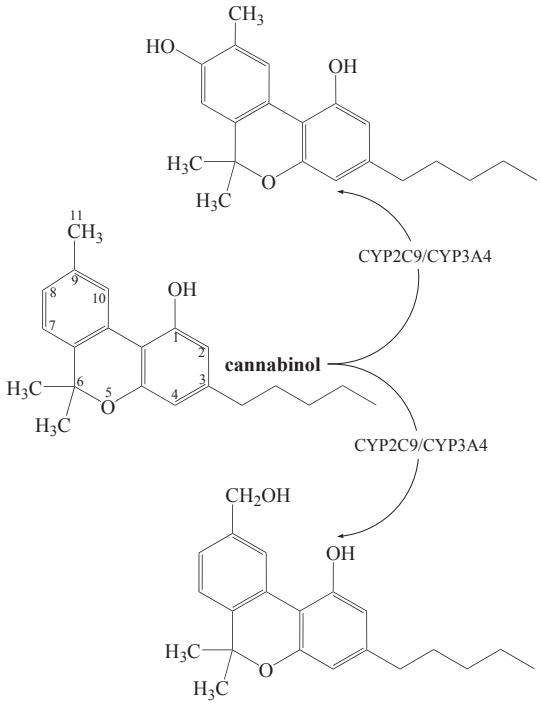

Fig. (6). Hydroxylation of CBN by CYP enzymes [69, 71].

2.2.1. Δ^9-tetrahydrocannabinol

The oxidative biotransformation of Δ^9-THC is quite complicated – approximately 80 metabolites were identified in humans [67]. The majority of the biotransformation processes of Δ^9-THC are catalyzed by CYPs (Fig. **5**). The first metabolite of Δ^9-THC was described back in 1970 by Nilsson *et al*., who used NMR to identify 11-hydroxy-Δ^9- THC in an extract from the incubation of a crude

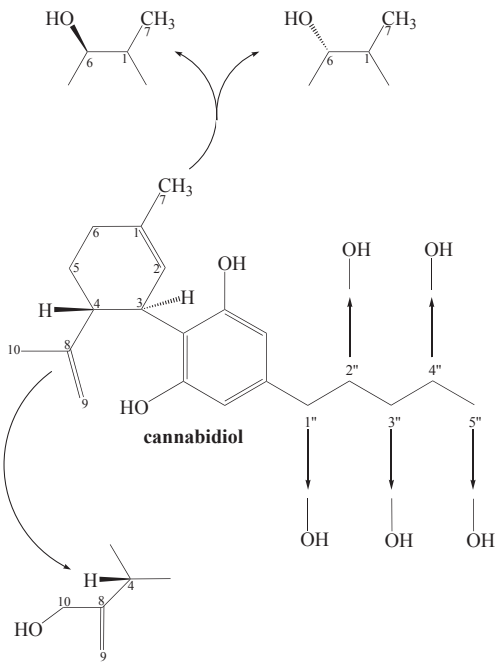

Fig. (7). Hydroxylation of CBD by CYP enzymes [72].

microsomal fraction of rabbit liver with Δ^9-THC [68]. This metabolite was formerly named 7-hydroxy-Δ^1-THC, because of the different numbering of the terpenophenolic ring in the past. The second most abundant hydroxy-derivative of Δ^9-THC is 8β-hydroxy-Δ^9-THC [69]. Later on, many other metabolites were identified, mostly in experiments with liver microsomes of different species, including humans, and the relative importance of CYPs was also examined, containing epoxygenated metabolites of THC [70]. The authors suggest that CYP2C9 and CYP3A4 probably play the most important roles in the oxidative metabolism of Δ^9-THC. Recently, Stout *et al*. [65] published a unique systematic review on the metabolism of cannabinoids.

Some of the metabolites of Δ^9-THC seem to be active (e.g. 11-hydroxy-Δ^9-THC) and therefore some authors think that the oxidative metabolism of Δ^9-THC may be necessary for the effects of cannabis [69].

2.2.2. Cannabinol

Cannabinol metabolism was studied by Kuzuoka *et al*. [71] and Watanabe *et al*. [69]. The chemical structure of CBN, being similar to Δ^9-THC, leads us to expect similar metabolic pathways mediated by microsomal monooxygenases. The hydroxylations occur at positions 8 and 11, and CYP2C9 and CYP3A4 are involved in their formation as reported in [65, 69, 71] (Fig. **6**).

2.2.3. Cannabidiol

The metabolism of cannabidiol was investigated both *in vivo* and *in vitro*. 33 different metabolites were found in human urine from a patient treated with CBD, 600 mg/day [72].

CBD is metabolized primarily by the enzymes CYP2C19 and CYP3A4 [65, 73]. The hydroxylation reactions occur at positions 6, 7, and positions 1″- 5″ of the aliphatic pentyl- and position 10 on the propenyl- substituent (Fig. **7**). Moreover, these metabolites may be further oxidized to form dihydroxylated metabolites and CBD-oic acid derivatives [72]. In an experiment with recombinant human liver microsomes, Jiang *et al*. proved that 7 out of 14 recombinant human CYP enzymes may be involved in CBD metabolism [73]. These include CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.

Among monohydroxylated metabolites, the most abundant were 6α-OH-, 6β-OH-, 7-OH-, 1″-OH-, 2″-OH-, 3″-OH-, 4″-OH-, and 5″-OH-CBD [73]. The authors also confirmed the importance of CYP3A4 and CYP2C19 in the overall metabolism of CBD, namely in the 6α-, 6β-, 7-, and 4″-hydroxylations of CBD with the use of selective isoform-specific inhibitors and anti-CYP3A4 antibodies.

2.2.4. Other Phytocannabinoids

The metabolism of other phytocannabinoids has not been studied in humans, but the hydroxylation of several cannabinoids, including THC, CBD, CBN, cannabichromene (CBC), and cannabigerol (CBG) was studied *in vitro* in the liver microsomal fraction in several animal species [74]. In general, similar hydroxylation reactions are catalyzed by microsomal fractions, but particular CYPs responsible for the reactions were not identified. Hydroxylation occurs most abundantly at the allylic part of the molecule at positions C5' and C6'. Apart from C5' and C6' hydroxylations, hydroxylation also occurs at positions C2' and C1'' to C5'', and epoxidation at the double bond of the methylpentenyl group [74] (Fig. **8**).

Cannabigerol metabolism appeared to be similar to the metabolism of CBC. Hydroxylations at the terminal allylic group of the side chain were the most abundant reactions in the liver microsomes of all species except for mouse, where C6' or C7' epoxide was the most abundant [74] (Fig. **8**).

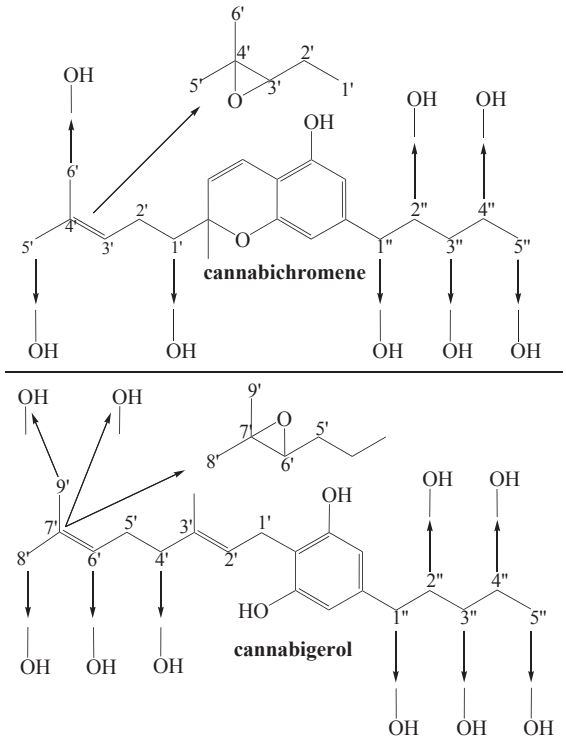

Fig. (8). Structure of CBC (**A**) and CBG (**B**) and positions of oxidative metabolism mediated by microsomal enzymes in mouse, rat, guinea pig, rabbit, hamster, gerbil, cat [74].

In summary, phytocannabinoids are extensively metabolized by CYP enzymes. For the most studied THC, CBN, and CBD, the enzymes CYP2C9, CYP2C19, and CYP3A4 catalyze the majority of hydroxylations. Most cannabinoids exhibit a similar pattern of oxidative metabolism [74]. At first, tricyclic cannabinoids (THC, CBD, and CBN) are the most effectively hydroxylated at the C-11 position and to a lesser extent also at the C-8 position. Various degrees of hydroxylation and epoxidation also occur at the carbons of the side chain in all cannabinoids, with the exception of CBN.

2.3. Synthetic Cannabinoids

Besides the substances isolated from natural materials, many other ligands of CB receptors were synthetized *in vitro*. Synthetic cannabinoids cover the whole spectrum of receptor ligand types from full agonists to inverse agonists, and their biological effects are therefore miscellaneous [75]. For the purposes of this review, synthetic cannabinoids are classified into groups of drugs for therapeutic purposes, molecules used as research tools, and abused drugs.

2.3.1. Synthetic Cannabinoids as Medicinal Products

Dronabinol (Marinol®) is a synthetic Δ^9-THC for oral use. It is approved for medical use in the United States and several other countries. Dronabinol is indicated for the treatment of anorexia associated with weight loss in patients suffering from AIDS and for the treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic therapy [76]. It undergoes extensive first-pass hepatic metabolism, primarily by microsomal hydroxylation via multiple CYPs, yielding both active and inactive metabolites. Its principal active metabolite is 11-OH-Δ^9-THC [76].

Nabilone (Cesamet®) is a synthetic THC analogue for oral administration. It is registered in Canada, the USA, and several other countries for the treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic treatments [77]. It has been suggested that the antiemetic effect of nabilone is mediated by its interaction with the cannabinoid CB1 receptor within the central nervous system. The metabolism of nabilone is extensive, and several metabolites have been identified. According to the data from Cesamet® SPC [77], there are at least two metabolic pathways involved in the biotransformation of nabilone. A minor pathway is initiated by the stereospecific enzymatic reduction of the 9-keto moiety of nabilone to produce the isomeric carbinol metabolite. Secondly, a metabolite of nabilone in faeces has been identified as a diol formed by reduction of the 9-keto group plus oxidation at the penultimate carbon of the dimethylheptyl side chain. In addition, there is evidence of extensive metabolism of nabilone by multiple CYPs. *In vitro* CYP inhibition studies using human liver microsomes showed that nabilone did not significantly inhibit the metabolic activity of CYP1A2, 2A6, 2C19, 2D6, and 3A4. In clinical use, nabilone is unlikely to alter the CYP-mediated metabolism of co-administered drugs [77].

Rimonabant (Acomplia®) was the first CB1 antagonist/inverse agonist to be approved for therapeutic use in metabolic syndrome and obesity [78]. Because of the significant risk of serious psychiatric adverse effects, it was withdrawn from the market [79]. *In vitro* experiments revealed CYP3A4 and amidohydrolase to be the major metabolic pathways involved in the biotransformation of rimonabant into inactive metabolites [80].

A buccal spray preparation containing *Cannabis* extracts, whose main active ingredients are Δ^9-THC and CBD (Sativex®), is now available in many countries including the UK, Spain, Italy, and Germany (not available in the US). It is used for the symptomatic relief of spasticity or neuropathic pain in multiple sclerosis and in cancer pain [81]. The active substances have the same structures as natural Δ^9-THC and CBD, therefore they undergo the same metabolic pathways.

2.3.2. Synthetic Cannabinoids as Experimental Tools

Compounds that are known to activate CB1 and CB2 receptors with approximately equal potency and that are most commonly used in the laboratory as CB1/CB2 receptor agonists fall essentially into one of four chemical groups: classical cannabinoids, nonclassical cannabinoids, amino-alkylindoles, and eicosanoids [29].

The classical group consists of dibenzopyran derivatives. The prototypic synthetic member of this group is HU-210, a synthetic analogue of (-)-Δ^8-THC. HU-210 displays a high affinity for CB1 and CB2 receptors, and also a high potency and relative intrinsic activity as a cannabinoid receptor agonist [29]. In the study of Kim *et al*. [82], the *in vitro* metabolism of HU-210 was investigated using human liver microsomes to characterize associated phase I metabolites. HU-210 was metabolized to yield a total of 24 metabolites, characterized as mono-oxygenated, mono-hydroxylated, di-oxygenated, or di-hydroxylated metabolites. The specific enzymes involved in the formation of the metabolites were not investigated.

The nonclassical group contains bicyclic and tricyclic analogues of Δ^9-THC that lack the pyran ring. The most widely used member of this group is CP 55,940. The oxidative metabolism of CP 55,940 was studied in mouse liver microsomes by Thomas *et al*. [83]. The mass spectral data indicated that five monohydroxylated metabolites had been formed differing in their position of hydroxylation. Two additional compounds were detected whose mass spectral data suggested that these metabolites were hydroxylated at two positions on the side chain. Side chain hydroxylation is consistent with the metabolic profile of Δ^9-THC [83].

The prototype of the aminoalkylindole group widely used in cannabinoid research is WIN 55,212-2. WIN 55,212-2 exhibits a relatively high efficacy at the CB1 and CB2 receptors and possesses CB1 and CB2 affinities in the low nanomolar range. The structure of WIN 55,212-2 bears no structural similarity to classical, nonclassical, or eicosanoid cannabinoids [84]. The metabolism of WIN 55,212-2 in rat liver microsomes was investigated in the study of Zhang *et al*. [85]. The HPLC chromatogram revealed two major and at least six minor metabolites derived from the parent compound. The two major metabolites (representing 60 to 75 % of the total metabolites) were each identified as dihydrodiol metabolites resulting from the arene oxide pathway. Three of the minor metabolites corresponded to structural isomers of the trihydroxylated parent compound, the other two represent monohydroxylated isomers and another was determined to be a dehydrogenation product. Specific enzymes involved in the formation of metabolites were not investigated.

Members of the eicosanoid group of cannabinoid CB1/CB2 receptor agonists have structures quite unlike those of classical, nonclassical, or aminoalkylindole cannabinoids. Two prominent members of this group are the endocannabinoids AEA and 2-AG.

The starting point for the development of the first CB1 selective agonists was the AEA molecule [29]. A number of agonists with significant selectivity for CB1 or CB2 receptors have been developed. Important CB1 selective agonists include the AEA analogues R-(+)-methanandamide, arachidonyl-2´-chloroethylamide (ACEA), and arachidonyl-cyclopropylamide (ACPA). Of these, both ACEA and ACPA share the susceptibility of AEA to enzymatic hydrolysis by FAAH [29, 86]. In contrast, methanandamide is less susceptible to enzymatic hydrolysis, probably because it is protected from this by the presence of a methyl substituent at the 1´ carbon [87].

The CB2 selective agonists most widely used as experimental tools have been the classical cannabinoid JWH-133, and the less selective aminoalkylindole JWH-015 [29]. The *in vitro* phase I metabolism of JWH-015 using human liver microsomes was studied by Mazarino *et al*. [88]. A total of 18 metabolites were formed. The biotransformation pathways detected consist of mono-hydroxylation, di-hydroxylation, tri-hydroxylation, carboxylation, N-dealkylation, dehydratation, and combinations of them, confirming data from the study with rat liver microsomes [89]. Specific enzymes involved in the oxidative metabolism were not studied.

2.3.3. Synthetic Cannabinoids as Abused Drugs

Synthetic cannabinoids recently became the largest group of compounds to be monitored in Europe by the EU Early Warning System on new psychoactive substances [90]. “Legal high” products containing synthetic cannabinoids (SCs) have probably been sold as herbal smoking mixtures since 2006. In 2008, a synthetic

cannabinoid JWH-018 was detected for the first time in a herbal mixture. In 2014, a further 30 new synthetic cannabinoids were reported for the first time, bringing the total number reported by the EU Early Warning System to 137 in February 2015 [91]. New drugs are synthesized by slight modifications of the known psychoactive "parent" compound, to obtain similar - or even stronger - psychoactive effects and to circumvent the law, being not yet included in the lists of controlled substances [92]. The common property of all SCs is that they interact with the CB1 and CB2 cannabinoid receptors and elicit cannabimimetic effects similar to Δ^9-THC. They are synthesized in clandestine laboratories and illegally added to commercial products such as herbal blends (these are sold under brand names such as "Spice" and "K2"), which are claimed to be air fresheners or herbal incenses. The most common way of administration is smoking.

The majority of compounds are chemically unrelated to Δ^9-THC. To date hundreds of SCs were categorized into the following structural groups: adamantoylindoles, aminoalkylindoles, benzoylindoles, cyclohexylphenols, dibenzopyrans, naphthoylindoles, naphthylmethylindoles, naphthylmethylindenes, naphthoylpyrroles, phenylacetyl-indoles, tetramethylcyclopropylketone indoles, quinolinyl ester indoles, and indazole carboxamide compounds [93].

Only limited data are available on the metabolism of the huge variety of synthetic cannabinoids. Due to the insufficient toxicity data, controlled human drug administration studies are not feasible. Therefore, *in vitro* experiments are alternative approaches for metabolite profiling and structure elucidation. Most of the recent *in vitro* metabolite-profiling studies utilize human liver microsomes or human hepatocytes. So far, we have identified such studies for the following synthetic cannabinoids: AB-CHIMINACA [94], AB-FUBINACA [95-97], AB-PINACA [95, 96, 98], 5F-AB-PINACA [98], ADB-FUBINACA [95], AKB-48 [99, 100], 5F-AKB-48 [100], AM-2201 [101, 102], APICA [103], CP 47,497 [104], HU-210 [82], JWH-015 [88], JWH-018 [105-108], JWH-073 [108], JWH-073 4-methylnaphthoyl analogue [108], JWH-122 [108-110], JWH-200 [109], JWH-210 [88], MAM-2201 [110], PB-22 [95, 111], 5F-PB-22 [95, 111], RCS-4 [112], RCS-8 [113], STS-135 (5F-APICA) [103, 114], UR-144 [102], and XLR-11 [115].

In vitro metabolite-profiling studies with subsequent confirmation in authentic specimens provide critically important information for the identification of suitable *in vivo* biomarkers to document the intake of SCs in clinical and forensic settings.

Two of the above-cited investigations also focused on the identification of specific CYP enzymes involved in oxidative metabolism. Chimalakonda *et al*. [101] studied the oxidative metabolism of [1-naphthalenyl-(1-pentyl-1H-indol-3-yl]-methanone (JWH-018) and its fluorinated counterpart AM-2201 [1-(5-fluoropentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone. Kinetic analysis using human liver microsomes and six human recombinant CYPs (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) identified CYP2C9 and CYP1A2 as the major CYPs responsible for the generation of hydroxylated and carboxylated metabolites of JWH-018 and AM-2201. The contribution of CYP2C19, 2D6, 2E1, and 3A4 in the hepatic metabolic clearance of these synthetic cannabinoids was minimal. These findings are further supported by the results of another investigation that observed a concentration-dependent inhibition of JWH-018 and AM-2201 oxidation in human liver microsomes by the CYP2C9- and 1A2-selective chemical inhibitors sulfaphenazole and α-naphthoflavone, respectively [116]. The study of Holm *et al*. [100] was focused on the elucidation of CYP enzymes involved in the oxidative metabolism of N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (AKB-48, also known as APINACA). Metabolite formation was screened using a panel of nine recombinant CYPs (CYP1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4) and compared the metabolites formed to human liver microsomal incubations with specific inhibitors against CYP2D6, 2C19, and 3A4, respectively. The results demonstrate that CYP3A4 is the major CYP responsible for the oxidative metabolism of AKB-48, preferentially performing the oxidation on the adamantyl moiety of the compound. Such detailed data are not available for other SCs. However, SCs are structurally diverse and the involvement of other CYPs and non-CYP enzymes in phase I biotransformations is likely. This was recently demonstrated for the quinolineindole synthetic cannabinoids PB-22, 5F-PB-22, and BB-22, where carboxylesterase 1 hydrolyzes an ester bond [96]. In addition, carboxylesterase 1 also hydrolyzes the primary amide group of two other synthetic cannabinoids, AB-PINACA and AB-FUBINACA [96].

Based on the recent evidence, synthetic cannabinoids are extensively metabolized in phase I and phase II biotransformation reactions. CYPs are involved in phase I metabolism. Oxidative metabolism forms preferably mono-, di-, and tri- hydroxylated, carboxylated, and N-dealkylated metabolites. Unlike Δ^9-THC metabolism, several SC metabolites retain a high affinity for the CB1 and CB2 receptors and exhibit a range of intrinsic activities. The majority of phase II SC metabolites are glucuronides.

3. CANNABINOIDS AS REGULATORS OF CYP METABOLIC ACTIVITY

The therapeutic use of cannabis, its extracts and synthetic cannabinoids together with the pleiotropic regulatory activity of the endocannabinoid system and the role of CYP in the metabolism of cannabinoids raises the question of drug-drug interaction with co-administered medicines. The decrease in the metabolic activity of individual CYPs can increase the plasma levels of their substrates, and symptoms of toxicity could appear. In the opposite direction, increased CYP activity will decrease the efficacy of its substrates, and can lead to the failure of a therapy.

The interactions between cannabinoids and CYPs could be simply caused by the competition of two substrates at the same CYP protein. Nevertheless, the huge metabolic capacity of CYP and involvement of alternative metabolic pathways decreases the clinical importance of such drug-drug interplay. The second possibility involves the direct interaction of cannabinoids with the CYP protein in a non-competitive (allosteric) manner, which usually leads to enzyme inactivation or a slowdown of the metabolic reaction. On the other hand, the possibility of allosteric induction for some substances was also described [117]. The final possibility for how cannabinoids can influence the CYP-mediated metabolism of co-administered drugs is by targeting the expression of CYP genes. This possibility is likely, due to the involvement of the endocannabinoid system in many physiological functions, including some metabolic pathways [118, 119], and its interaction with many other neuronal systems and circuits which might also be involved. Therefore, the results of research focused on the direct interaction of cannabinoids with CYP enzymes and the influence of cannabinoids on the metabolic activity assessed after systemic administration or in models using living cells with intact signal pathways could give different results and are described separately.

3.1. Direct Interactions of Cannabinoids and CYP Proteins

Rimonabant was tested for the ability to bind to CYPs in the model of human liver microsomes (HLM). Approximately 19 % of the metabolites produced were covalently bound to CYPs [120]. Therefore it is not surprising that a mechanism-based inhibition of CYP3A4 and decrease in the metabolic activity over 70 % was described. A search for other CB1 antagonists for possible therapeutic use led to the synthesis of aminopyrazine CB1 inverse agonists. The chlorine in the para position of the 5-phenyl ring was found to be responsible for the inhibition of CYP3A4 and its substitution with a trifluoromethyl moiety did not change the potency at the CB1 receptor, increased aqueous solubility, and decreased potential for CYP3A4 inhibition [121]. The negative psychotropic effects of rimonabant could be eliminated with analogues not crossing the blood-brain barrier but with the effect on peripheral CB

receptors. LH-21, a CB1 antagonist with poor brain penetration, is similar to rimonabant in both its anorectic affect and also CYP inhibiting activity [122]. It inhibits the metabolic activity of CYP3A4, 2C9, and 2D6 with the IC_{50} of 1.62, 8.14, and >105 μM, respectively. The inhibition was weaker in comparison to the control inhibitors ketoconazole, sulfaphenazole, and quinidine. LH-21 is reported to be a moderate inhibitor of CYP3A4 and CYP2C9 and weak inhibitor of CYP2D6.

Ashino *et al*. described the inhibition of the CYP1A2 metabolic activity of different synthetic cannabinoids with the indole structure moiety in the model of mouse liver microsomes [123]. MAM-2201 and JWH-019 with the naphtoylindole structure were the most potent inhibitors, and decreased the activity to 47.7 % and 64.3 % of the control values at a concentration of 100 μM. Most of the adamantoylindole derivatives inhibited the activity weakly (up to a 10 % decrease) except for STS-135, which exhibited an inhibition comparable to naphtoylindole ligands. The last molecule tested with the tetramethylcyclopropylindole core exhibited moderate inhibitory activity with a decrease to 73.4 % of the control values at a 100 μM concentration.

Δ^9-THC, CBN, and CBD are the most studied substances of the phytocannabinoids group in terms of CYP interactions. All of them competitively inhibit the CYP1A enzyme family, but with different strengths [124]. The most potent inhibitor of CYP1A1 is CBD followed by CBN, while the inhibition of CYP1A2 and CYP1B1 was stronger after CBN treatment. All the enzymes were inhibited by Δ^9-THC less potently, with a low selectivity for individual CYP1 enzymes. The subsequent studies revealed the pentylresorcinol [125] or methylresorcinol [126] structures to be important for the direct inhibition of CYP1A1. The same moiety is probably involved in the potent CYP2B6 inhibition by CBD [127], while Δ^9-THC and CBN have a weaker effect. All of the substances decreased the activity in a mixed fashion in comparison to the inhibition of CYP2A6, which is non-competitive, and the inhibition potency of all three phytocannabinoids was weaker than the inhibition of CYP2B6. Similar results were obtained with CYP2C9, which was also inhibited by all three substances [128]. The strongest inhibition was reported for CBN, followed by Δ^9-THC and CBD. The same substances were incubated with rat liver microsomes, and the 16α- and 2α–hydroxylation of testosterone was assessed [129]. The reaction is mediated by CYP2C11, which is considered to be the counterpart of human CYP2C9 [130]. However, the results are different from those obtained in the human studies. The inhibition was only detected in CBD-treated samples, while Δ^9-THC and CBN did not influenced the enzyme activity. Both CBN and Δ^9-THC decreased the omeprazole 5-hydroxylase activity, indicating their inhibitory effect on CYP2C19 [131], and at least one of the free phenolic groups and pentyl side chain are the structural determinants of this effect. The activity of CYP2D6 is again most sensitive to the effect of CBD [132]. Its IC_{50} values were 2-4 times lower than those of Δ^9-THC and CBN. The CYP2D6 inhibition potency of these two is similar. Similarly to the influence on the enzymes of the CYP1A family, CYP3A enzymes are differentially sensitive to the effect of the major phytocannabinoids. CBD inhibited the activity of CYP3A4 and CYP3A5 most potently, while the influence of all three substances on the activity of CYP3A7 was comparable [133]. The inhibition of 3A4 can also be indirectly evidenced by the suppression of cyclosporine A metabolism in both mouse and human liver microsomes preincubated with CBD [134]. The effect of phytocannabinoids on the activity of 17α-hydroxylase (CYP17) was tested in the model of rat testis microsomes. However, CBD was the most potent inhibitor of CYP enzymes in most of the documented experiments, its inhibitory effect on the CYP17 activity was the weakest, and required IC_{50} concentrations over 290 μM [135]. On the other hand, Δ^9-THC and CBN inhibited the enzyme's activity with EC_{50} values of 42.8 μM and 32.9 μM, respectively. The inhibition of individual CYPs by phytocannabinoids is in accordance with the older data obtained with less selective CYP substrates [136-138].

The clinical relevance of the presented data is questionable, due to the high concentrations of the tested drugs used in *in vitro* studies and their correspondence to plasma levels reached when phytocannabinoids are used therapeutically or abused. When a marijuana cigarette (15.8 mg Δ^9-THC) is smoked, the peak plasma concentrations of Δ^9-THC is reported to be only 268 nM [139]. Similarly after CBD and CBN (20 mg) administration by smoking a cigarette, the levels reached 363 nM and 406 nM, respectively [140, 141]. Moreover, the plasma levels of synthetic Δ^9-THC dronabinol reached a nanomolar concentration when administered in the recommended therapeutic doses [124]. The review of inhibition constants (Ki) values are presented in Table **5**. It is obvious that clinically relevant inhibition of CYP by phytocannabinoids is likely for enzymes of the CYP1 family with CBN and for the CYP1A1, 2B6, 2C19, and 3A5 enzymes with CBD. The inhibition of CYP enzymes by THC is probably too weak to cause a clinically significant interaction with the co-administered drugs.

3.2. The Influence of Cannabinoids on CYP Metabolic Activity – *In vivo* and Cell Culture Models

The possible discrepancy in the results of direct interaction experiments and the systemic administration of drugs can be demonstrated in the work of Bornheim *et al*. [142]. Different analogues of THC were tested for both direct interaction with naïve mouse liver microsomes, and microsomes sampled 2 hours after the systemic administration of THC analogues to mice. While in the direct interaction part of the study, some of the tested substances inhibited the activities of CYP3A and CYP2C, the same molecules produced no effect after systemic drug administration.

An important factor influencing the result of the study is the duration of drug pre-treatment before the activity is assessed. The results after a single dose of a drug and after the repeated administration can be different. After the repeated administration of a drug, higher values of plasma/tissue concentrations can be reached than with a single dose. Moreover, there is probably interplay between cannabinoids and endocannabinoid CB receptors, which can lead to changes in signal pathways including CYP liver regulation mechanisms. The subsequent change could therefore be time-dependent, such as for instance the induction of the enzyme activity by the mechanism of increased gene transcription, and *de novo* protein synthesis usually takes at least several hours from the drug administration. An example can be found in the study concerning the effect of the synthetic cannabinoid receptor agonist CP 55,940 on CYP activity in rats [143]. The only parameter that changed after a single intraperitoneal dose of the drug (0.4 mg/kg) was an increase in the oxygen consumption by the brain and liver. However after 11 days of treatment with the same dose of the substance the increased brain and hepatic mitochondrial respiration disappeared, and the P-450 reductase, benzo(A)pyrene hydroxylase, and ethoxycoumarin deethylase activities as well as the protein content of the liver microsomes were increased.

The results of the *in vivo* experiment undoubtedly also depend on the experimental model used. When CP 55,940 was administered to mice (intraperitoneally, 0.5 mg/kg/day) for 5 or 24 days, the microsomal protein content was decreased after the latter type of administration [144], in contrast to the previous results with the same substance in rats. Nevertheless, the activity of CYP2E1, measured as p-nitrophenol oxidation, was unaffected. These data correspond with the results of Yang *et al*. from HepG2 cells incubated with a natural CB receptor agonist CBD [145]. Other researchers reported an increase in the expression of CYP2E1 and CYP2C6, together with an increased amount of total CYP hepatic content in mice after a single dose or repeated administration of hashish [146].

Table 5. Inhibition of CYP metabolic activity *in vitro* [124, 125, 127-129, 131-133, 135].

	1A1	1A2	1B1	2A6	2B6	2C9[1]	2C11[2]	2C19	2D6[1]	3A4	3A5	3A7	17[3]
CBD	0.16[C]	2.69[C]	3.63[C]	55.0[N]	0.69[M]	5.6[C]	19.9 - 21.6[C]	0.793[M]	2.42	1.0[C]	0.195	12.3[C]	124.4[M]
Δ^9-THC	4.78[C]	7.54[C]	2.47[C]	28.9[N]	2.81[M]	1.5[M]	none	1.93[M]	17.1[A]	>50[A]	35.6[A]	30.3[A]	15.9[M]
CBN	0.54[C]	0.08[C]	0.15[C]	39.8[N]	2.55[M]	0.93[C]	none	no data	12.3[A]	>50[A]	>50[A]	23.8[A]	4.5[M]

[A]Half maximal inhibitory concentration (IC_{50}) in μM. Other reported values are inhibition constants (Ki) in μM; [M]Mixed type of inhibition; [C]Competitive type of inhibition; [N]Non-competitive type of inhibition; Enzymes are recombinant human proteins, if not indicated different; [1] Human liver microsomes.; [2] Rat liver microsomes; [3] Rat testis microsomes.

The variability in the experimental design thus leads to a high variation in the results obtained. However, if the data from the experiments with the same designs are compared, the variability disappears. The first data regarding the influence of phytocannabinoids on the activity of CYP in the animal models were homologous. An extract from cannabis prolonged the sleeping time of mice treated with the CYP substrate pentobarbitone, documenting the inhibition of its metabolism [147]. Similar results were obtained with CBD [148], the most studied cannabinoid in terms of CYP interactions. Moreover, CBD inhibited the enzyme activities of various more or less selective CYP substrates including p-nitroanisole O-demethylase [137], aniline hydroxylase [137], hexobarbital hydroxylase [149], erythromycin N-demethylase [149], 6β-testosterone hydroxylase [149], and aminopyrine N-demethylase [150].

The influence of CBD on single CYPs was evaluated in a number of studies. CBD administered to mice at a dose of 120 mg/kg inactivated CYP2C and CYP3A proteins by covalent binding after a single dose [151]. Whereas after repeated administration of the same dose, the expression of the mRNA of these two enzymes increased as well as the protein content, while the activity remained unchanged [152], probably because of the inactivation reported previously. Similarly, in rats CBD decreased the total CYP hepatic content after repeated administration [153]. Further research revealed decreased activities of CYP17 and CYP2C [154]. The influence of CBD on CYP2C activity is probably also sex-dependent [155].

The most recent results from human cell cultures described the induction of CYP1A enzymes by marijuana smoke [156, 157], Δ^9-THC [157-159], and CBD [159], probably mediated by the aryl hydrocarbon receptor (AhR) [157, 159].

One of the great advantages of animal models is the possibility of studying the effects of prenatal exposure to drugs. Maternal exposure to Δ^9-THC, CBD, and CBN increased the levels of hepatic CYP content, whereas postnatal exposure had the opposite effect in male rat offspring [160].

3.3. Drug-Drug CYP-Mediated Interactions of Cannabinoids

The interspecies differences in the CYP system [161] creates a great barrier to the clinical approximation of data obtained from animals. Nevertheless, in most preclinical studies either with Δ^9-THC or CBD, the results correspond to *in vitro* experiments, and generally these cannabinoids are reported to be CYP inhibitors. CBD significantly decreased the metabolism of CYP substrates, e.g. cocaine [162], anandamide [163], cyclosporine A [134], or THC [162, 164, 165]. However, the dose of CBD necessary to evoke the effect was 30 mg/kg in mice, which is higher than any dose of CBD used in clinical practice.

Although there is enough evidence of the influence of cannabinoids on the total hepatic amounts of CYPs and their activities from preclinical studies, the clinical data on the topic are scarce. The risk of interaction is significantly dependent on the dose administered. Rimonabant (40 mg/day for 8 days) did not affect the steady-state concentration of co-administered digoxin, midazolam, warfarin, nicotine or oral contraceptives [166]. The effect of medicinal cannabis (Bedrocan®), containing 18 % Δ^9-THC and 0.8 % CBD administered for 15 days, on the levels of irinotecan and docetaxel were tested in oncologic patients [167]. Similarly, no significant change in the clearance or exposure to the monitored drugs was observed. Finally, the summary of medicinal product characteristics of the synthetic Δ^9-THC and CBD mixture (Sativex®) declares that no interactions with CYP3 substrates are expected [168]. It has to be stressed, that these results describe the risk of interaction of low doses of CBD. Nadulski *et al*. tested the effect of 5.4 mg of CBD on the pharmacokinetics of 10 mg of Δ^9-THC [169] and concluded that the inhibitory effect of CBD on CYP in this dose is small compared to the variability of CYP activity caused by other factors. This conclusion could be generalized for the clinical use of CBD at doses of up to 5 mg per day. On the other hand, higher doses of CBD in the range 8-25 mg/kg/day were described to markedly inhibit the metabolism of hexobarbital [170] or clobazam [171], both CYP3A4 substrates. The interaction potential of the higher doses of CBD with CYP3A4 substrates is therefore clinically relevant.

4. POSSIBLE INVOLVEMENT OF ENDOCANNABINOID SYSTEM IN THE REGULATION OF CYP EXPRESSION AND CYP METABOLIC ACTIVITY IN THE LIVER

The regulation of CYP metabolic activity is complex in nature, including many endogenous and exogenous factors determining the actual amounts of enzymes and their catalytic activities. Besides the exogenous ones, genetic polymorphisms, and the role of hormones are known to be endogenous factors regulating the expression and activity of CYPs. Recently, the involvement of some neuronal systems was reported [172-174]. The regulatory role of the endocannabinoid system raises the question of its participation in this process, too. Here, we hypothesize that the central and peripheral pathways of the endocannabinoid system and interplay between cannabinoid ligands and various receptors are probably involved in CYP regulation.

4.1. The Role of Central Endocannabinoid System in the CYP Regulation

It is known that genes coding for various CYPs are regulated by endogenous hormones, which are under the control of the central nervous system. It has been also shown that changes in the brain dopaminergic, noradrenergic, and serotonergic systems can affect hepatic CYP expression [175]. The central endocannabinoid system modulates neurotransmission at inhibitory and excitatory synapses, and therefore could be also involved in the regulation of CYP activity. Thus the endocannabinoid system and possible interactions with other neuronal systems, its impact on the hypothalamic-pituitary axis (HPA) and on the levels of circulating hormones are reviewed.

4.1.1. The Brain and Endocannabinoid System

Most central cannabinoid effects are mediated by the CB1 receptors widely expressed throughout the brain, where they are the most abundant in regions controlling a number of key functions [30, 176]. Therefore, CB1 receptors are present at a high density in the basal ganglia, frontal cortex, hippocampus, and cerebellum, and at a

moderate/low density in the nucleus accumbens, hypothalamus, and amygdala [177].

The predominant localization of CB1 receptors at the presynaptic terminals of neurons plays an important regulatory role, because they can influence the release of a number of different neurotransmitters [178, 179]. The postsynaptic localization of CB1 receptors has been also observed, but only rarely [177]. The endocannabinoids are synthesized and released by postsynaptic neurons, and they act as retrograde neuronal messengers at presynaptic CB1 receptors. The activation of CB1 receptors by endocannabinoids suppresses the presynaptic release of γ-aminobutyric acid (GABA), glutamate, acetylcholine, serotonin, and noradrenaline [179].

Another reason for the increased complexity of endocannabinoid signaling is the evidence that CB1 receptors form heteromers with a variety of other GPCRs [180]. CB1 receptors can form functional heterodimers with μ-opioid receptors [181], orexin-1 receptors [182, 183], adenosine A_{2A} receptors [184, 185], serotonergic (mainly 5-HT_3) receptors [186, 187], or dopaminergic D_2 receptors [188]. Another possible interaction between the dopaminergic and endocannabinoid system is indirectly via the GABAergic [189, 190] and glutamatergic system [191, 192].

Endocannabinoid signaling in the brain may influence liver CYP activity, but the signal has to be somehow mediated from the CNS to the periphery. It is known that such signal transduction could be found in hormones released from HPA and leading to subsequent changes in the hormonal levels released from peripheral organs. Hormones influence not only hepatic glucose or lipid metabolism, but also the expression of genes coding for different CYP liver enzymes [193-196].

Dopaminergic pathways which could possibly contribute to the release of hormones are the mesolimbic and the tuberoinfundibular pathways. It was reported that stimulation of the dopaminergic system increases growth hormone (GH) [197, 198], adrenocorticotropic hormone (ACTH), and corticosterone levels [199, 200]. In contrast, the levels of thyroid-stimulating hormone (TSH) after activation of the dopaminergic system were decreased [198, 201]. The ability of the brain dopaminergic system to affect liver CYP expression by altering the levels of pituitary hormones was first reported in the studies of Wójcikowski *et al*. [174, 202]. Dopaminergic D_2 receptors were identified to be involved in the regulation of hormones and liver CYP enzymes in the mesolimbic pathway [174].

Noradrenaline is one of the main neurotransmitters controlling the release of GH [172, 203]. It also controls the production and release of corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) [204]. The release of somatostatin is regulated by noradrenaline, among other neurotransmitters and neuropeptides. It was reported that a damage of the noradrenergic innervation in the arcuate nucleus (ARC) or periventricular hypothalamic nucleus (PVN) proved an opposite effect on the regulation of CYP expression [172]. This can be explained by the fact that destruction of the noradrenergic innervation in the ARC leads to a decrease of the noradrenaline level and also to a decrease of the level of GH. While destruction of the noradrenergic innervation in the PVN causes a decrease of the level of noradrenaline, but the plasma concertation of GH are increased probably due to the decreased secretion of somatostatin. The involvement of the noradrenergic system in CYP regulation was confirmed by Kot *et al*. [205]. Again the hormones were identified to be the mediators of the effect from the brain to the liver. The same author reported the serotonergic system to also take part in CYP neurohumoral regulation [173, 206, 207].

The influence of exogenous cannabinoids on the secretion of pituitary hormones has been known for a long time, but the role of endocannabinoids in the neuroendocrine system is not fully understood yet [208, 209]. Based on available studies, two options for how endocannabinoids influence HPA are suggested: (i) a direct effect mediated by endocannabinoids receptors and/or (ii) an indirect effect when cannabinoids change the activity of the endocannabinoid system and this modulates the activity of other neuronal systems controlling HPA. CB1 receptors are expressed in various regions of the brain, and were also detected in the hypothalamus and pituitary gland [210, 211]. Many studies describe the influence of cannabinoids or endocannabinoids on the levels of CRH, GH, TSH, prolactin (PRL), and luteinizing hormone (LH), but their findings are often contradictory [208, 212-216]. It seems that the main structure of endocannabinoid influence on neuroendocrine functions is the hypothalamus, where they act as retrograde messengers activating the CB1 receptors. Importantly, it was revealed that endocannabinoids are involved in the rapid negative feedback actions of glucocorticoids (GCs) in parvocellular neurons of the hypothalamic paraventricular nuclei (PVN) containing CRH. GCs, after binding to glucocorticoid (GR) receptors localized in the PVN, activate the postsynaptic GPCRs. This leads to the synthesis and release of endocannabinoids. These endocannabinoids act as retrograde messengers to the CB1 receptors located at presynaptic glutamate terminals and inhibit glutamate release [217]. These findings thus provide a possible mechanism for the rapid feedback inhibition of the hypothalamic pituitary adrenal axis by GCs. Moreover, the CB1 receptors and endocannabinoids are found throughout all of the extrahypothalamic sites that regulate PVN neuronal activation, such as the hippocampus, prefrontal cortex, amygdala, bed nucleus of the stria terminalis, and midbrain monoaminergic nuclei, such as the locus coeruleus and dorsal raphe [218]. These brain regions are the most likely sites of interaction between the endocannabinoid system and other nervous systems [190, 219-221].

Once the CB ligands directly change the activity of the endocannabinoid system or indirectly the activity of other neurotransmitters and the HPA is changed, hormones start the signal transduction towards the liver (Fig. **9**). The regulation of liver CYP enzymes by hormones involves binding the hormone to the nuclear receptor and translocation of the ligand-receptor complex into the cell nucleus. The expression of specific genes including CYP enzymes is activated or inhibited. GH, GCs, and TSH are ligands of nuclear receptors able to change the expression of CYP genes [222-224]. The influence of hormones on the transcription activity of CYP genes is described at a glance in Table **6**.

4.2. The Involvement of Peripheral Cannabinoid Receptors in the CYP Regulation

When administered systemically, cannabinoids are able to target both the regulation centers in the brain and the receptors in peripheral tissues including the liver. Except for the direct interaction with CYPs (see chapter 3.1.) there is a possibility of the influence of cannabinoids on the receptors of target cells. The receptor specificity of cannabinoids is broad due to their high structure variability, therefore there are many receptors which might be activated or inhibited with regard to the properties of the ligand. Here we describe the evidence of interaction between cannabinoids and peripheral receptors involved in the signal pathways of CYP regulation and the role of these receptors in CYP regulation.

The key ligand-activated transcriptional regulators of CYPs are the pregnane X receptor (PXR), constitutive androstane receptor (CAR), retinoid X receptors (RXRs), peroxisome proliferator-activated receptors (PPARs), glucocorticoid receptors (GRs), and aryl hydrocarbon receptor (AhR) [222].

Briefly, PXR plays a key role in the regulation of the CYP2B6, CYP2C, CYP2A6, CYP3A, and CYP4F12 genes [238-245]. Agonists of PXR induce these CYP enzymes. In addition to the induction of CYP enzymes, PXR activation also represses CYP7A1 expression as a protective feedback in response to the accumulation of bile acids in the liver [224, 246].

Table 6. Hormonal regulation of CYP genetic transcription [175, 193-195, 225-237].

GC	T3	GH	PRL
↑2A6[b]	↓1A1/2[a]	↑1A2[b]	↓2C11[a]
↑2B6[b]	↓2A1/2[a]	↑2A1[a]	↓2D1[a]
↑2B8[b]	↓3A1/2[a]	↑2C7[a]	↓3A4[a]
↑2C8[b]	↓3A4[b]	↑2C12[a]	
↑2C9[b]	↑7A1[b]	↑2C11[a]	
↑2C19[b]		↓2C19[b]	
↑3A4[b]		↑2D1[a]	
↑3A5[b]		↓2E1[a]	
		↑3A1[a]	

[a]Data from preclinical experiments on rats. [b]Data obtained from human cell lines.

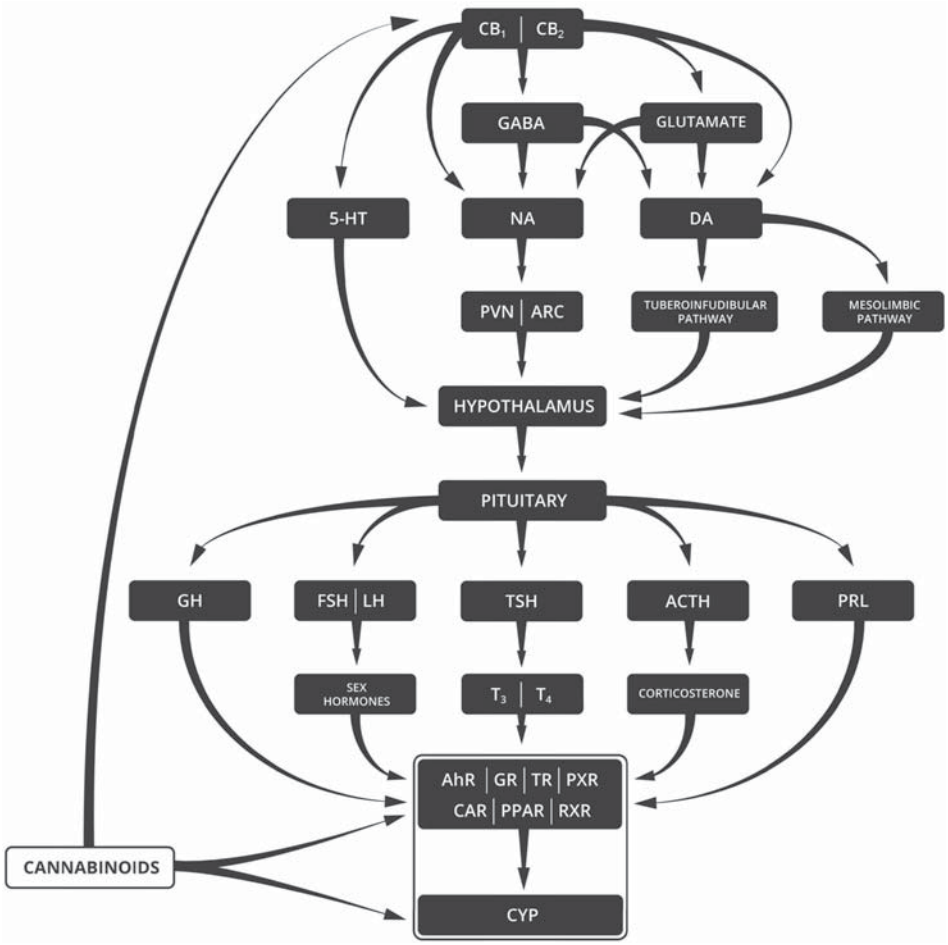

Fig. (9). Endocannabinoid system and cannabinoids in the regulation of CYP activity.

The activation of CAR is linked to the induction of CYP2B [247]. There is signaling cross-talk between PXR and CAR. These receptors control the expression of human CYP2A6 [238], CYP2B6 [248], CYP2C8/9 [230, 249], CYP2C19 [232], CYP3A4 [250], CYP3A5 [251], and CYP3A7 [252].

AhR is not a true nuclear receptor; it belongs to a family of transcription factors that contain the basic-helix-loop-helix and Per-ARNT-Sim domains. AhR requires the AhR nuclear translocator as its heterodimerization partner to be translocated into the nucleus and turn on the CYP gene transcription. AhR triggers the expression of CYP1A and CYP1B [222, 223].

The PPAR family currently has four members - PPARα, PPARβ, PPARγ, and PPARδ - which differ in their localization [253]. After activation by appropriate ligands, PPARs bind as heterodimers with RXR (PPAR/RXR) to peroxisome proliferator response elements. It has been shown that CYP2B, CYP3A, and

Table 7. Cannabinoid ligands of nuclear receptors [157, 159, 257, 258].

PPARα	PPARγ	PPARδ	AhR
N-oleoylethanolamine	AEA, 2-AG	N-oleoylethanolamine	Δ^9-THC
palmitoylethanolamide	NADA, Δ9-THC		CBD
virodhamine	ajulemic acid		
noladin ether	CP 55,940, HU-210		
	WIN 55,212-2		

CYP4A are activated by PPAR, and CYP2C11 is suppressed by the PPAR agonist [254, 255].

CYP3A and CYP2B proteins are distinctly regulated by GRs [256]. GRs may induce the expression of a gene that does not contain GRE in its promoter. This is exerted by indirect "trans-regulation". Moreover, GRs contribute to functional cross-talk between the PXR, CAR, AhR, and RXR signaling pathways [222, 226].

RXR is directly or indirectly involved in the regulation of many enzymes and can be considered to be a limiting factor in the overall regulation of hepatic gene expression patterns [253].

Some endocannabinoids, phytocannabinoids, and synthetic cannabinoids are ligands of different PPARs and the AhR. An overview of the selectivity of drugs to individual receptors is given in Table **7**.

5. CONCLUSION

Cannabinoids are a structurally and pharmacodynamically heterogeneous group of drugs with great potential for therapeutic use in the near future. The involvement of CYP in their metabolism is clear and indisputable, whereas the clinical significance of their drug-drug interactions has yet to be evaluated in detail. These interplays may have various mechanisms from the direct interaction of two substrates at the same enzyme, through different types of antagonism with the CYP protein to the activation of various receptors and changes in hormonal levels leading to an alteration in the expression of CYP genes. The latter describes the suggested involvement of the endocannabinoid system in the central regulation of hepatic CYP activity. This hypothesis is based on indirect evidence, and could be proved or refuted by further studies. Moreover, we suggest that changes in liver CYP metabolic activity could be time-dependent. Our idea is based on the signal transduction from the brain to the liver via hormones which are under HPA control, and a negative feedback mechanism plays a significant role here. Therefore, the hormonal changes induced by drug administration can be short-lived as well as the changes in CYP activity. To the best of our knowledge, the factor of time was not studied in any of the previous works focusing on the role of the central nervous system in the regulation of liver CYP activity.

LIST OF ABBREVIATIONS

Δ^9-THC	=	(-)-trans-Δ^9-tetrahydrocannabinol
2-AG	=	2-arachidonoylglycerol
5-HT	=	5-hydroxytryptamine, serotonin
AA	=	Arachidonic acid
ABHD	=	α,β-hydrolase domain
ACEA	=	Arachidonyl-2′-chloroethylamid
ACPA	=	Arachidonyl-cyclopropylamide
ACTH	=	Adrenocorticotropic hormone
AhR	=	Aryl hydrocarbon receptor
ARC	=	Arcuate nucleus
CAR	=	Constitutive androstane receptor
CBC	=	Cannabichromene
CBD	=	Cannabidiol
CBG	=	Cannabigerol
CBN	=	Cannabinol
CYP	=	Cytochrome P450
DA	=	Dopamine
diHETE	=	Dihydroxyeicosatrienoic acids
EET	=	Epoxyeicosatrienoic acids
EET-EA	=	Epoxyeicosatrienoic acid ethanolamide
EET-G	=	Epoxyeicosatrienylglycerol derivatives
FAAH	=	Fatty acid amide hydrolase
GABA	=	γ-aminobutiric acid
GCs	=	Glucocorticoids
GH	=	Growth hormone
GPCRs	=	G-protein coupled receptors
GRs	=	Glucocorticoid receptors
HEET-EA	=	20-hydroxy-epoxyeicosatrienyl ethanolamide
HETE	=	Hydroxyeicosatrienoic acid
HETE-DA	=	Hydroxyeicosatetraenyl dopamine
HPA	=	Hypothalamic-pituitary axis
LH	=	Luteinizing hormone
NA	=	Noradrenaline
NADA	=	N-arachidonoyldopamine
PPARs	=	Peroxisome proliferator-activated receptors
PRL	=	Prolactin
PVN	=	Paraventricular nucleus
RXRs	=	Retinoid X receptors
SC	=	Synthetic cannabinoid
T3	=	Triiodothyronine
T4	=	Thyroxine
TSH	=	Thyroid-stimulating hormone

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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