Pharmacological exploitation of the endocannabinoid system: new perspectives for the treatment of depression and anxiety disorders?

Exploração farmacológica do sistema endocanabinoide: novas perspectivas para o tratamento de transtornos de ansiedade e depressão?

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Abstract

Objective: The present review provides a brief introduction into the endocannabinoid system and discusses main strategies of pharmacological interventions. **Method:** We have reviewed the literature relating to the endocannabinoid system and its pharmacology; both original and review articles written in English were considered. **Discussion:** Cannabinoids are a group of compounds present in Cannabis sativa (hemp), such as Δ^9-tetrahydrocannabinol, and their synthetic analogues. Research on their pharmacological profile led to the discovery of the endocannabinoid system in the mammalian brain. This system comprises at least two G-protein coupled receptors, CB_1 and CB_2, their endogenous ligands (endocannabinoids; e.g. the fatty acid derivatives anandamide and 2-arachydonoyl glycerol), and the enzymes responsible for endocannabinoid synthesis and catabolism. Endocannabinoids represent a class of neuromessengers, which are synthesized on demand and released from post-synaptic neurons to restrain the release of classical neurotransmitters from pre-synaptic terminals. This retrograde signalling modulates a variety of brain functions, including anxiety, fear and mood, whereby activation of CB_1 receptors was shown to exert anxiolytic- and antidepressant-like effects in preclinical studies. **Conclusion:** Animal experiments suggest that drugs promoting endocannabinoid action may represent a novel strategy for the treatment of depression and anxiety disorders.

Descriptors: Cannabis sativa; Cannabinoids; Endocannabinoids; Anxiety; Depression

Resumo

Objetivo: *Este artigo revisa o sistema endocanabinoide e as respectivas estratégias de intervenções farmacológicas.* ***Método:*** *Realizou-se uma revisão da literatura sobre o sistema endocanabinoide e a sua farmacologia, considerando-se artigos originais ou de revisão escritos em inglês.* ***Discussão:*** *Canabinoides são um grupo de compostos presentes na* Cannabis sativa *(maconha), a exemplo do Δ^9-tetraidrocanabinol e seus análogos sintéticos. Estudos sobre o seu perfil farmacológico levaram à descoberta do sistema endocanabinoide do cérebro de mamíferos. Este sistema é composto por pelo menos dois receptores acoplados a uma proteína G, CB_1 e CB_2, pelos seus ligantes endógenos (endocanabinoides; a exemplo da anandamida e do 2-araquidonoil glicerol) e pelas enzimas responsáveis por sintetizá-los e metabolizá-los. Os endocanabinoides representam uma classe de mensageiros neurais que são sintetizados sob demanda e liberados de neurônios pós-sinápticos para restringir a liberação de neurotransmissores clássicos de terminais pré-sinápticos. Esta sinalização retrógrada modula uma diversidade de funções cerebrais, incluindo ansiedade, medo e humor, em que a ativação de receptores CB_1 pode exercer efeitos dos tipos ansiolítico e antidepressivo em estudos pré-clínicos.* ***Conclusão:*** *Experimentos com modelos animais sugerem que drogas que facilitam a ação dos endocanabinoides podem representar uma nova estratégia para o tratamento de transtornos de ansiedade e depressão.*

Descritores: *Cannabis sativa; Canabinoides; Endocanabinoides; Ansiedade; Depressão*

Introduction

Because of its analgesic, antiemetic and tranquilizing effects, the herb *Cannabis sativa* has been used for medical purposes for centuries. In addition, preparations of cannabis, such as marijuana, hashish or skunk, have a long history as drugs of abuse.[1] Typical

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effects of cannabis abuse are amnesia, sedation and a feeling of well-being described as "bliss".[2] In the middle of the last century, Raphael Mechoulam and colleagues identified Δ^9-tetrahydrocannabinol (Δ^9-THC) as the main psychoactive ingredient of this herb. Today, it is known that *Cannabis sativa* contains more than 60 substances, such as cannabidiol, cannabinol and cannabicromene, which are referred to as phytocannabinoids.[3] Their lipid nature posed a significant obstacle to chemical experiments, which might explain why the discovery of phytocannabinoids occurred late compared to other natural compounds (e.g. morphine was isolated from opium in the XIX century). The molecular structure rendered it likely that Δ^9-THC exerts its effects primarily by changing physico-chemical characteristics of cell membranes. Therefore it came as a surprise that specific binding sites could be identified within the mammalian brain,[4] followed by isolation and characterization of endogenous binding substances, named endocannabinoids.[5] The development of novel pharmacological compounds targeting receptors or ligand synthesis and degradation revealed a number of complex brain functions, which are tightly controlled by the endocannabinoid system. The aim of the present review is to briefly introduce this system and its pharmacology, to discuss its involvement in psychopathology and to illustrate its therapeutic potential.

Method

We have reviewed the literature relating to the endocannabinoid system and the possibilities of pharmacological interventions in this system. Original studies employing animals or humans subjects and review articles written in English were considered.

Discussion

1. The endocannabinoid system of the brain

The endocannabinoid system comprises the receptors, the endogenous agonists and the related biochemical machinery responsible for synthesizing these substances and terminating their actions. The receptors were named by the International Union of Basic and Clinical Pharmacology (IUPHAR) according to their order of discovery as CB_1 and CB_2 receptors.[6] Both are G-protein coupled receptors. Within the central nervous systems, CB_1 is primarily localized at presynaptic nerve terminals and accounts for the majority of neurobehavioural effects of cannabinoids. CB_2, in contrast, is the major cannabinoid receptor in the immune system, but may also be expressed in neurons. The main endogenous agonists of CB_1 and CB_2 are arachidonic acid derivates. Arachidonoyl ethanolamine was the first endocannabinoid characterized and nicknamed anandamide, after the Sanskrit *ananda*, meaning "bliss".[5] Later on, 2-arachydonoyl glycerol (2-AG) was also identified,[7] followed by N-arachidonoyl dopamine (NADA), 2-arachidonoyl-glycerol ether (noladine) and O-arachidonoyl ethanolamine, also termed virodhamine.[8] Endocannabinoids may bind to receptors other than CB_1 and CB_2, for instance to the transient receptor potential vanilloid type-1 (TRPV1), formerly the "capsaicin receptor" or "vanilloid receptor" (VR1), an ion channel. In the peripheral nervous system, TRPV1 is activated by heat, low pH and the red chilli pepper substance capsaicin.[9] Within the central nervous system, TRPV1 is expressed in postsynaptic nerve terminals and might be activated intracellularly by anandamide. Other endocannabinoid receptors are the formerly "orphan" G-protein coupled receptor 55 (GPR55) and the peroxisome proliferator activated receptors (PPAR). Furthermore, an allosteric site at the CB_1 receptor has been identified, which may provide an interesting target for pharmacological intervention.[10]

2. Modes of endocannabinoid action

Classical neurotransmitters such as acetylcholine, amino acids (e.g. glutamate, GABA) or monoamines (e.g. dopamine, serotonin) fulfil the following criteria: 1) The transmitters are synthesized in pre-synaptic terminals from specific precursors and stored in synaptic vesicles. 2) They are released into the synaptic cleft after calcium influx. 3) There are specific mechanisms to terminate their actions, including uptake and enzymatic degradation.[11,12] These criteria render endocannabinoids atypical messengers, which mediate information transfer from post- to pre-synaptic terminals in a retrograde manner: Endocannabinoids are synthesized on-demand and not stored in vesicles. The synthesis occurs in post-synaptic neurons following calcium influx and subsequent activation of phospholipases (phospholipase D in the case of anandamide and diacyglycerol lipase in the case of 2-AG), which convert membrane phospholipids into endocannabinoids.[13] They seem to immediately reach the synaptic cleft by free or assisted diffusion and to bind to presynaptically localized CB_1 receptors.[14] Via a complex network of intracellular signalling processes, activation of CB_1 receptors finally results in decreased calcium influx into the axon terminals and, thus, to down-regulation of transmitter release. Other than CB_1, activation of TRPV1 receptors by anandamide leads to increased depolarisation of postsynaptic membranes. Therefore, activation of CB_1 and TRPV1 seem to exert opposing effects.

As is the case for some classical neurotransmitters, the actions of endocannabinoids are limited by a two-step process: internalization followed by catabolism.[15] The first step remains elusive, since it is still a matter of debate whether internalization of endocannabinoids occurs passively via diffusion or by specific transporters.[16-19] After internalization, endocannabinoids undergo enzymatic hydrolysis. The primary enzymes responsible for anandamide and 2-AG hydrolysis are fatty acid amide hydrolase (FAAH)[20] and monoacylglyceride lipase (MGL),[21] respectively. Intriguingly, the two endocannabinoids are degraded either pre- (2-AG) or post-synaptically (anandamide). Both FAAH and MGL have emerged as important pharmacological targets with promising therapeutic potential. Figure 1 summarizes our current knowledge about the major "players" of the endocannabinoid system.

3. Pharmacological manipulation of the endocannabinoid system

Several pharmacological tools have been developed that interfere with the endocannabinoid system. Some may act directly at CB_1 or CB_2 receptors (i.e., agonists or antagonist). Others may act in

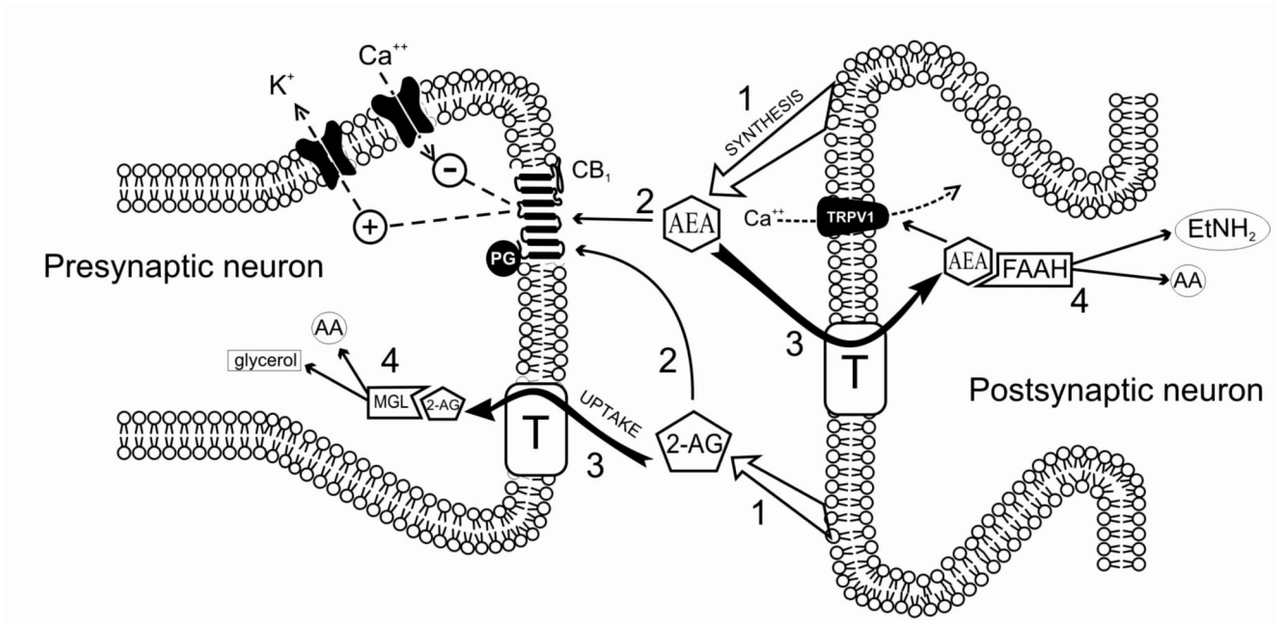

Figure 1 - Schematic representation of endocannabinoid action. Endocannabinoids are synthesised in and released from the membrane of post-synaptic neurons on-demand after calcium influx (1). They activate pre-synaptic CB_1 receptors and restrain neural activity (2). Anandamide and 2-AG are removed from the synaptic cleft by up-take into the post- and pre-synapse, respectively (3). Once inside neurons, anandamide binds TRPV1 (with consequences opposite to those of CB_1 activation) and undergoes hydrolyse by FAAH, whereas 2-AG is hydrolysed by MGL (4).

a more indirect manner, e.g. by interfering with mechanisms that terminate endocannabinoid action. Table 1 lists representative examples for each of the intervention strategies, which will be introduced in the following paragraphs.

1) Cannabinoid receptor agonists

Based on the chemical structure of Δ^9-THC, several synthetic agonists have been developed with diverse intrinsic activities and affinities for cannabinoid receptors.[6,22] In this context the mouse tetrad emerged as a valuable tool for characterization of CB_1 receptor agonists. The tetrad stands for four main effects of systemic cannabinoid treatment: hypolocomotion, catalepsia, hypothermia and analgesia.[23,24] Studies in conditional knockout mice with cell type-specific deletion of CB_1 revealed that the tetrad effects are mediated by different neuronal populations.[25]

Some agonists show the same affinity for CB_1 and CB_2 receptors, such as Δ^9-THC, nabilone, WIN-55,212-2, CP-55940 or HU-210. Others bind rather selectively to CB_1 (e.g. ACEA) or CB_2, (e.g. AM-1241, JWH-133). In addition, compounds acting on the allosteric site of CB_1 have been developed (e.g. Org275796, Org29647 and PSNCBAM).[10] Apart from Δ^9-THC, other phytocannabinoids with low affinity for CB_1 receptor (e.g. cannabidiol) may act through complex mechanisms, targeting receptors not related to the endocannabinoid system.[26-28]

2) Enhancement of endocannabinoid action

Drugs that enhance endocannabinoid action may provide a more subtle strategy for pharmacological interventions than direct activation of cannabinoid receptors. Given that endocannabinoids are produced and released on-demand, compounds interfering with endocannabinoid uptake and degradation could increase CB_1 signalling with temporal and neuroanatomical specificity. Such drugs are expected to induce fewer side-effects compared to direct agonists, as will be discussed later. A number of drugs have been developed that seemingly increase endocannabinoid action by blocking endocannabinoid uptake.[17,29] Examples are AM404, VDM11, UCM707, OMDM and AM1172. Drawbacks of these compounds are that they may lack pharmacological selectivity, in addition to targeting, with the endocannabinoid transporter a still elusive biochemical entity.

Another strategy to increase endocannabinoid signalling is to inhibit catabolic processes. This approach appears to be the most promising, since the enzymes responsible for endocannabinoid hydrolysis are well characterized. Among the FAAH-inhibitors, URB-597 has been most widely studied so far.[30,31] This compound irreversibly blocks FAAH with good target selectively, leading to increased anandamide levels. More recently, inhibitors of MGL have been developed as well (e.g. URB602 or JZL184), which cause increased bioavailability of 2-AG.[32,33] Inhibition of 2-AG, but not anandamide, hydrolysis exerts tetrad effects similar to CB_1 agonists.[33] This underscores the functional dissociation of 2-AG and anandamide action.

3) Inhibition of endocannabinoid action

Several antagonists have been synthesized with different affinities for CB_1 and CB_2 receptors. The first and prototype compound that binds to the CB_1 receptor and blocks the effects of its endogenous ligands is SR141716A (SR1; rimonabant).[34] Another widely employed CB1 antagonist is AM25.[6,22] CB_2 receptors, in turn, can be blocked by SR1414528 and AM630 in a selective manner.[6,22]

Table 1 - Compounds interfering with the endocannabinoid system: potential advantages and drawbacks (for references, see text)

Target	Pros (+)	Cons (-)
CB_1/CB_2 agonists Δ^9-THC nabilone, CP-55940, HU-210, WIN-55,212-2 **CB_1 agonists** ACEA, R-(+)-methanandamide	- anxiolytic-/antidepressant-like properties - some already in clinical use in specific circumstances: anti-emetic, appetite-stimulant, analgesic properties	- may induce aversive states (particularly in higher doses), sedation, amnesia, tolerance, withdrawal syndrome, addiction
CB_1 allosteric agonists Org275796, Org29647	- might induce more subtle actions, with fewer side-effects as compared to conventional CB1 agonists	n.d.
CB_1 antagonists Rimonabant (SR141716A), AM251	- potentially useful in the treatment of addiction - beneficial effects on obesity, diabetes, cardiovascular disorders (metabolic syndrome)	- may facilitate the occurrence of anxiety and depression
CB_2 agonists AM-1241, JWH-133	- avoid the problems of CB1 activation - analgesic properties	n.d.
CB_2 antagonists SR1414528, AM630	n.d.	n.d.
TRPV1 agonists Capsaicin, resiniferatoxin, olvanil	- analgesic properties in the peripheral nervous system	- may facilitate anxiety
TRPV1 antagonists Capsazepine, iodo-resiniferatoxin, SB366791	- anxiolytic-/antidepressant-like properties - may act by redirecting anandamide-actions exclusively towards CB1	n.d.
Endocannabinoid-uptake inhibitors AM404, VDM11, UCM707, OMDM, AM1172 **FAAH inhibitors** URB597, MAFP	- anxiolytic-/antidepressant-like properties - take advantage of on-demand actions of endocannabinoids, possibly with fewer side-effects as compared to CB1 agonists - low potential for addiction - take advantage of on-demand actions of anandamide (FAAH-inibitors)	- molecular target remains elusive (endocannabinoid transporter) - some drugs lack selectivity - long-term effects have been poorly investigated - at higher concentrations, anandamide may also activate TRPV1, thereby potentially occluding CB1-mediated favourable effects
Dual FAAH/TRPV1 blockers AA-5HT	- same anxiolytic effects as pure FAAH inhibitors, but with higher efficacy	n.d.
MGL inhibitors URB602, JZL184	- take advantage of on-demand actions of 2-AG	- contrary to FAAH inhibitors, may mimic some typical effects of CB1 agonists (tetrad)
Endocannabinoid-synthesis inhibitors O-3640, O-3841	n.d.	n.d.

n.d. – not determined / remains to be investigated

An alternative strategy to reduce endocannabinoid signalling would be by inhibiting anabolic enzymes. So far, this strategy has not been widely explored, possibly because of the diversity of mechanisms responsible for anandamide and 2-AG synthesis. First compounds which may inhibit 2-AG synthesis are O-3640 and O-3841.[35]

4. Role of the endocannabinoid system in psychiatric disorders

Rimonabant was the first pharmacological compound which interfered with the endocannabinoid system to be approved for the treatment of metabolic syndrome. Today we know that the drug exerts its beneficial effects primarily by blocking CB_1 receptors in the periphery. However, because of its lipophilic nature, rimonabant could cross the blood-brain barrier and get into the central nervous system. Here it had devastating side effects in patients, such as increase in depression, suicidality and anxiety disorder.[36] After being turned down by the FDA, rimonabant (also known as Accomplia™) has been retracted from market by Sanofi-Aventis. The rimonabant saga illustrates how clinicians learnt by "accident" that the plethora of anxiogenic effects described for the compound in animal models also applied to human beings. They might have been "warned" before by the dramatic effects of cannabis abuse on the regulation of emotional states: cannabis consumption may induce anxiolytic, euphoric and rewarding effects, in addition to improving mood.[2] However, in addition, psychotic symptoms, panic attacks and mood disturbances were frequently encountered after chronic cannabis consumption.[2]

Animal studies have provided more direct evidence for involvement of the endocannabinoid system in anxiety and depression. They revealed that the endocannabinoid system is functional in several brain regions, such as the prefrontal cortex, hippocampus, amygdala and midbrain periaqueductal gray,[37] that are involved in diverse psychiatric disorders. Moreover, mutant mice lacking expression of CB_1 receptors exhibit a plethora of behavioural changes that resemble stress-related psychopathology.[38] For instance, they show an anxiety-like phenotype in exploration based tests,[39,40] sustained fear responses,[41] impaired stress-coping[40,42] and impaired extinction of aversive,[43] but not appetitive,[44] memories. Treatment of wild-type mice with CB_1 receptor antagonists revealed essentially the same phenotypes.

Changes in endocannabinoid levels were consonant with the behavioural data. For instance, a variety of stressors caused an increase in endocannabinoid levels in the amygdala[43] or periaqueductal gray.[32] At the same time they reduced them in other structures, such as the hippocampus.[45] Divergent regulation of anandamide vs. 2-AG synthesis and tonic vs. phasic changes illustrate the complexity of those processes. Changes in endocannabinoid signalling within the hypothalamus[46] may contribute to the modulatory consequences of the endocannabinoid system on regulation of hormonal stress responses.[47]

Few studies have measured the levels of endocannabinoids in psychiatric disorders so far: basal serum concentrations of AEA and 2-AG were significantly reduced in women with major depression,[48] suggesting a role for this system in this disorder. Furthermore, schizophrenic patients show increased anandamide levels in the cerebrospinal fluid.[49] However, because of the complexity of intracerebral endocannabinoid signalling mentioned before, endocannabinoid measurements in blood and even cerebrospinal fluid samples might be of limited value for our understanding of the involvement of the endocannabinoid system in mood disturbances.

Taken together, with a few exceptions,[50,51] the majority of the preclinical and clinical data support a scenario, where attenuated endocannabinoid signalling promotes the occurrence of anxiety- and depression-like symptoms.

5. Pharmacological and therapeutic perspectives

The diverse substances that interfere with the endocannabinoid system and CB_1 signalling have been extensively studied in animals in terms of efficacy and side-effects in mood and anxiety regulation. The following paragraphs discuss the advantages and limitations of each of the treatment strategies (for summary see Table 1).

1) Cannabinoid receptor agonists

Low doses of Δ^9-THC and its synthetic analogues exerted anxiolytic-like effects in animal models of generalized anxiety disorder.[52] Furthermore, cannabinoids impaired the formation but facilitated the extinction of contextual fear.[53,54] Apart from anxiolytic-like activities, cannabinoids showed antidepressant-like properties. At the behavioural level, they alleviated the consequences of inescapable stressors in animal models of depression.[55,56] Moreover, cannabinoids increased the levels of neurotrophins, induced hippocampal neurogenesis and suppressed stress hormone secretion.[38,42,48]

Although one could envisage therapeutic applications for these substances, there are major obstacles that limit their applicability in clinical practice. For instance, cannabinoid treatment may cause addiction and tolerance, induce sedative effects, and impair learning and memory. In general, low doses tend to induce anxiolysis, whereas higher doses may induce opposite effects.[57,58] The reasons for these differences remain to be determined. They might be attributed to dose-dependent actions upon different brain regions and neural populations.[58] Moreover, high cannabinoid concentrations may lead to desensitization/ internalization of CB_1 receptors, thus resulting in decreased endocannabinoid signalling. It is tempting to assume that such processes account for the paradoxical effects of cannabis consumption on emotional responses such as episodes of anxiety and panic.[2] To circumvent these problems, future studies may try to target the allosteric site of the CB_1 receptor.[10]

2) Compounds that enhance endocannabinoid action

The major difference between the action of endogenous and exogenous cannabinoids is the on-demand activation of the endocannabinoid system in a temporally and spatially restricted manner. Drugs that enhance endocannabinoid action have been extensively studied in animal models of anxiety and depression. For instance, blockade of endocannabinoid up-take by AM404 induced anxiolytic-like effects[59,60] and facilitated the extinction of conditioned fear.[61,62] Also the treatment with the anandamide-hydrolysis inhibitor URB597 exerted anxiolytic-like effects similar to benzodiazepines.[30,60,63-65] URB597 showed also antidepressant-like actions in animal models of stress-related psychopathology.[66,67] Noteworthy, URB597 increased the activity of monoaminergic neurons projecting from the brain stem to the prefrontal cortex, an effect similar to those observed after chronic treatment with antidepressant drugs.[67]

It is of note that some well-established pharmacological compounds, such as aspirin or paracetamol, depend for their action at least partially on endocannabinoid signalling.[68] This may contribute their mood-lifting effects.[69]

In summary, anandamide uptake and/or hydrolysis represent promising pharmacological targets for the development of novel therapeutic strategies of depression and anxiety disorders. The effects induced by these "endocannabinoid-enhancers" differ from those of direct CB_1 agonists in several aspects: first, they avoid ubiquitous receptor activation, but promote endocannabinoid action in a temporally and spatially restricted manner. Second, they show a broader therapeutic window. Third, pre-clinical studies point to a significantly lower risk of addiction, abuse liability and tolerance. Fourth, the occurrence of biphasic paradoxical effects on emotional responses was less evident.

The applicability of "endocannabinoid-enhancers" is limited by promiscuous binding capabilities of anandamide, For instance, binding to TRPV1 seems to exert opposing effects to those

mediated via CB_1.[57,70] Hence, the simultaneous blockade of FAAH and TRPV1 may represent a reasonable approach to obtain more effective anxiolytic and/or antidepressant drugs. In fact, the compound arachidonoyl serotonin (AA-5HT), which meets those objectives, induced anxiolytic-like effects in mice with higher efficacy than URB597.[64]

3) Cannabinoid receptors antagonists

The development of novel generations of CB_1 receptor antagonists with restricted access to the brain may enable the exploitation of the beneficial effects of blocked endocannabinoid signalling in peripheral tissues (e.g. hepatocytes or adipocytes) on diabetes and metabolic syndrome in absence of the devastating side effects on mood and cognition.[36]

Conclusion

Malfunctions in the endocannabinoid system may promote the development and maintenance of psychiatric disorders such as depression, phobias and panic disorder. Thus, CB_1 agonists or inhibitors of anandamide hydrolysis are expected to exert antidepressant and anxiolytic effects. Future studies should consider 1) the development of CB_1 antagonists that cannot readily cross the blood-brain barrier, 2) shifts in the balance of CB_1 vs. TRPV1 signalling, 3) the allosteric site of CB_1 receptor and 4) the potential involvement of CB_2 receptor in mood regulation. Striking similarities in (endo)cannabinoid action in animals and men render it likely that the new pharmacological principle outlined in the present article may find their way into clinical practice.

Acknowledgments

F.A.M. is a recipient of a research grant from Fundação de Apoio à Pesquisa do Estado de Minas Gerais (FAPEMIG). C.T.W. is recipient of research grants from the Max Planck Society.

Disclosures

Writing group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
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Note: UFMG = Universidade Federal de Minas Gerais; MPI-Psychiatry = Max Planck Institute of Psychiatry; FAPEMIG = Fundação de Apoio à Pesquisa do Estado de Minas Gerais.
For more information, see Instructions for authors.

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