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The role of FAAH and MAGL inhibitors in anxiety disorders

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ABSTRACT

Anxiety disorders are currently one of the most common diseases in industrialized countries, showing a continuous upward trend. Classic therapy was based on GABA receptor modulation. However, numerous side effects associated with the impact on this neurotransmission led to the search for new drugs. The involvement of other messengers like serotonin, dopamine and noradrenaline in the pathophysiology of anxiety disorders, resulted in the introduction of SSRI, SNRI, TLPD and azapirones. However, these drugs are not fully effective, have many limitations and side effects, hence the problem of finding new solutions in anxiety therapy is still valid. One of new theories indicates the role of the endocannabinoid system in the diagnosis and treatment of anxiety. It is well known, that the main endogenous cannabinoids – anandamide (AEA) and 2-arachidonoglicerol (2-AG) – are metabolized by the enzymes from the serine hydrolase group: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). This review presents a new compounds form the froup of endocannabinoid degradation inhibitors with anxiolytic activity.

Keywords: anxiety disorder, endocannabinoids, FAAH inhibitors, MAGL inhibitors

1. INTRODUCTION

Anxiety is a psychological, physiological, and behavioral state, but may also be considered as adaptive component in response to a life-threatening situation. However, there is a difference between normal stress and pathological disorder. Anxiety disorders are one of the most common diseases in the world and according to epidemiological studies, they often comorbid with other mental disorders and are associated with a high economic burden. The number of patients suffering from this disease is constantly growing. The causes of its formation are known only to a small extent, due to the complexities of etiology, including neurobiological, genetic and psychosocial factors [1]. Anxiety disorders occur in many forms such as phobias, separation anxiety, generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD). Moreover, there is often a self-medication problem when patients reach for substances that give an apparent relief in sickness. Therefore, taking of alcohol or other substances of abuse is very common and may lead to the development of addiction and paradoxically increase the symptoms of the disease. The use of drugs from these groups may lead to interactions with anxiolytics [1].

There are various neurotransmitters that are involved in anxiety such as norepinephrine (NA), serotonin (5-HT), glutamate or gamma-amino butyric acid (GABA). Therefore classic pharmacotherapy of anxiety is based on the modulation of different neurotransmitters [2]. Currently, the most commonly used to treat anxiety disorders are selective serotonin reuptake inhibitors (SSRI) and selective serotonin-norepinephrine reuptake inhibitors (SNRI). However, the limitation in their use is fact, that therapeutic effect becomes apparent after about 2 weeks. Additionally, tricyclic antidepressants (TCA) are also used. They interact through a large number of receptors involved not only in anxiety but also other physiological processes, therefore their use is associated with numerous side effects [3].

Azapirones constitute a separate group of anti-anxiety drugs. They are used to enhance the effect of SSRI, since they interact with 5-HT1A receptors and increase serotonin concentration in the synaptic cleft. Moreover, azapirones may also act as antagonists of dopaminergic receptors (particularly D3, D4) involved in anxiety behavior. The exact mechanism of action of azapirones is still not fully explained and they are not widely used in pharmacotherapy [4].

Furthermore, benzodiazepines show rapid anxiolytic effect and for years they were used as drug of choice for treating anxiety disorders. However, the presence of numerous side effects such as sedation, muscle disorder, susceptibility to abuse and synergistic effects with alcohol and CNS depressants determines the need to look for new anxiolytics as effective as benzodiazepines, but without side effects. At the same time, drugs from other therapeutic groups have anxiolytic potential [3], however, efforts are still being made to explore the involvement of other mechanisms.

One of new theories indicates the role of the endocannabinoid system in the diagnosis and treatment of anxiety [5]. It is well known, that the main endogenous cannabinoids – anandamide (AEA) and 2-arachidonoglicerol (2-AG) – are metabolized by the enzymes from the serine hydrolase group: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Therefore the aim of this article was the characteristics of the endocannabinoid system and review of the new compounds form the group of endocannabinoid degradation inhibitors with anxiolytic activity.

2. MATERIALS AND METHODS

A literature search was performed using PubMed, Google Scholar, Scopus and Web of Science databases. The following terms were searched: "FAAH", "MAGL", "endocannabinoids", "anxiety" and limited to the English or Polish language.

3. CANNABINOIDS AND ENDOCANNABINOID SYSTEM

Cannabis sativa is a plant with more than 400 chemicals, over 100 of which are pharmacologically active phytocannabinoids [1]. It has long been used to rheumatism, malaria, constipation and childbirth and operational pain relief. These chemical compounds were recommended for analgesic, antiemetic and anticonvulsant use [6]. The main compound Δ-9-tetrahydrocannabinol (THC), with psychotropic effect, is a partial agonist of the cannabinoid (CB) receptor. At low doses, THC induces anxiolytic effect, but in high doses, it can cause anxiety. Moreover, it produces side effects like motor impairments, catalepsy, hypothermia and cognitive impairments [2,7].

The second most abundant, non-psychoactive compound found in *Cannabis sativa* is cannabidiol (CBD). Recent research proves that CBD has anxiolytic properties, but the exact mechanisms are still being studied [8].

The identification of phytocannabinoids led to the search for endogenous targets for these compounds which resulted in the discovery of endogenous cannabinoid system. To produce the effects, cannabinoids appear to activate specific endocannabinoid receptors, mainly in the central nervous system (CNS). The two primary and also most studied are CB1 and CB2 receptors [9]. Both types are G-protein-coupled receptors, negatively regulating adenylyl cyclase (which in turn inhibits cAMP) and positively - mitogen active protein kinase (MAPK).

Table 1. Location of CB_1 and CB_2 receptors in the brain.

Type of receptor	CB_1	CB_2
Location	CEREBRAL CORTEX BASAL GANGLIA HIPPOCAMPUS CEREBELLUM SPLEEN TONSILS GASTROINTESTINAL TRACT UTERUS PROSTATE VASCULAR SMOOTH MUSCLE CELLS ADRENAL GLANDS	SPLEEN MICROGLIAL CELLS Immune cells: NEUTROPHILS MACROPHAGES MONOCYTES LYMPHOCYTES

The distribution of CB receptors is different in areas of the brain: CB_1 is located in the CNS and CB_2 is mainly present in peripheral nervous system and immune cells. The brain areas important for cognition, emotional regulation, defensive behaviors like nucleus of stria terminalis, striatum, hypothalamus, periaqueductal grey, midbrain serotonergic and adrenergic nuclei contain high densities of these receptors (Table 1) [1, 10].

Binding of the ligand to the CB receptor results in:

1. Inhibition of adenylyl cyclase activity
2. Inhibition of Ca^{2+} channels
3. Activation of focal adhesion kinase (FAK) and MAPK
4. Reduction of the K^+ channels phosphorylation
5. Stimulation of additional intracellular pathways [9].

The activity of CB receptors can be regulated by AEA and 2-AG, the most bioactive endogenous specific cannabinoid substances with lipophylic structure (Fig. 1) [9, 13].
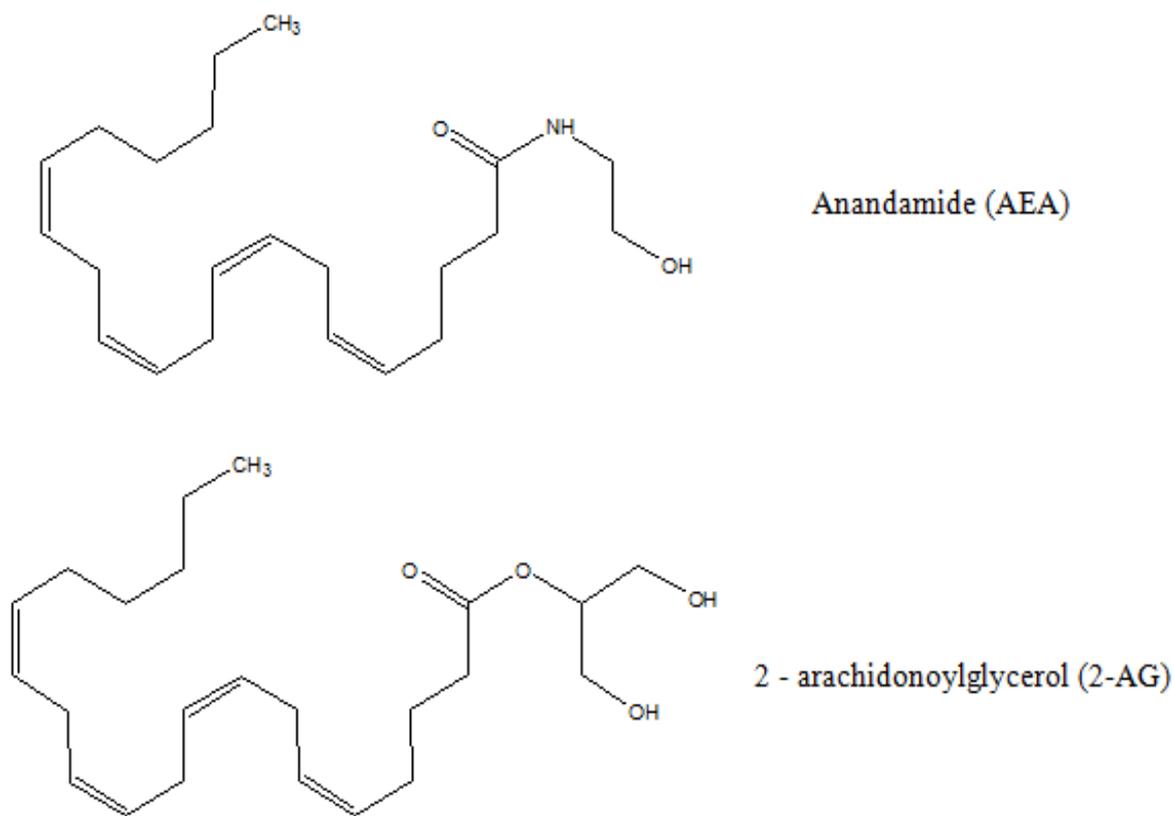

Figure 1. Structure of AEA and 2-AG

The AEA is synthesized from phosphatidylethanolamine by N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) at postsynaptic sites in regions including the basolateral amygdala. Synthesis reaction of 2-AG occurs with phospholipase C, which hydrolyzes phosphatidylinositol to diacylglycerol (DAG) and with

diacylglycerol lipase into 2-AG [5]. Endogenous cannabinoids are released from membrane phospholipids precursors and transported to the intracellular space with endocannabinoid membrane transporter (EMT) [6, 9].

Activation of the CB receptor by AEA and 2-AG, prevents excessive activity of neurons in the CNS. Endocannabinoid signaling is characterized by the fact that the synthesis of neurotransmitters occurs on-demand, after activation of the CB receptor [13]. Decreased endocanabinoid transmission caused by low levels of AEA and 2-AG produces anxiety. Therefore, the strategy for increasing endcannabinoid level has become the subject of research on anxiolytic processes [14]. Behavioral studies has shown that exposure to stress increases FAAH levels in some mice brain structures – mainly in the amygdala, which is responsible for anxiety reaction. It causes reduction of AEA level and activation of the hypothalamus - pituitary gland - adrenal gland axis (HPA), release of catecholamines in the brain and anxiety-like behavior [15, 17].

Expression of CB_1 receptors on the glutaminergic neurons of hippocampus plays a key role in adaptation to anxiety. Stress induces the release of glutamate from synaptic cleft but 2-AG has the ability to regulate this neurotransmission. Genetically modified animals with overexpression of MAGL in the hippocampus had decreased 2-AG level and showed excessive activation of glutaminergic signaling, which manifested as anxiety-like behavior [16].

The activity of endocannabinoids is regulated by specific enzymes. AEA can be reduced in hydrolytic pathway with FAAH as well as oxidative pathway with cyclooxygenase-2, lipooxygenase (5-, 12-, 15-,) and cytochrome P450 monooxygenase. FAAH degrades AEA into arachidonic acid and ethanolamine [11]. Hydrolysis of 2-AG is catalyzed mainly by MAGL into glycerol and arachidonic acid (Fig. 2) [6, 12].
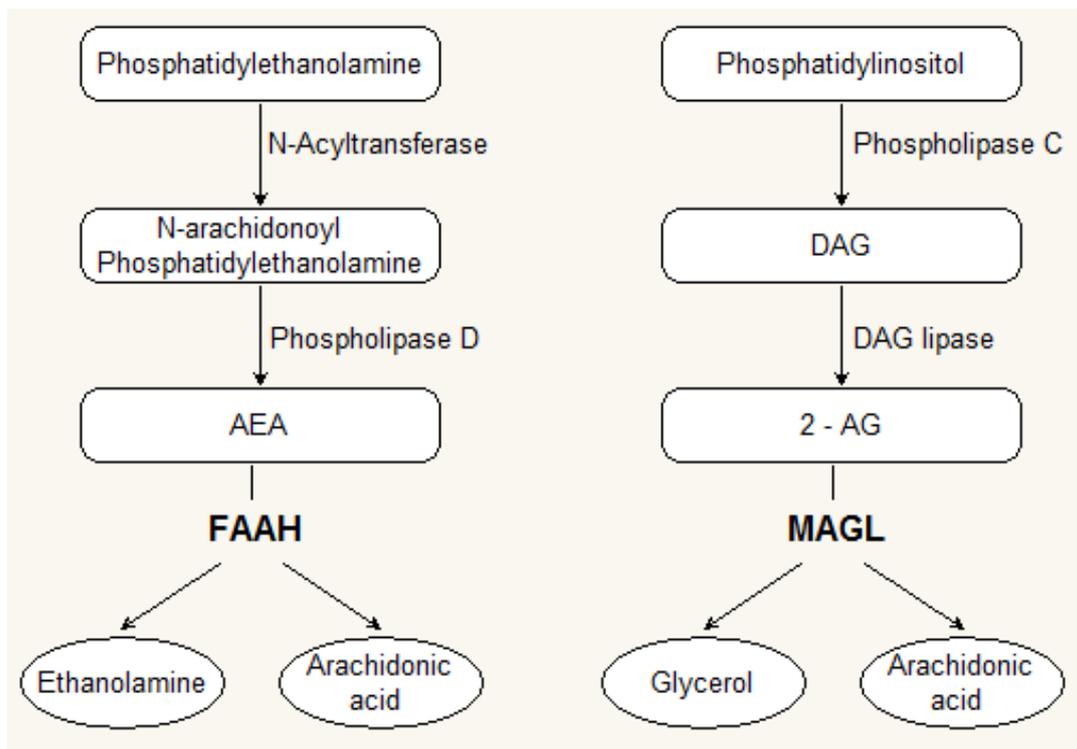

Figure 2. Synthesis and degradation of AEA and 2 – AG [40].

The CB receptors, together with enogenous ligands, and the enzymes involved in their synthesis and metabolism form endocannabinoid system.
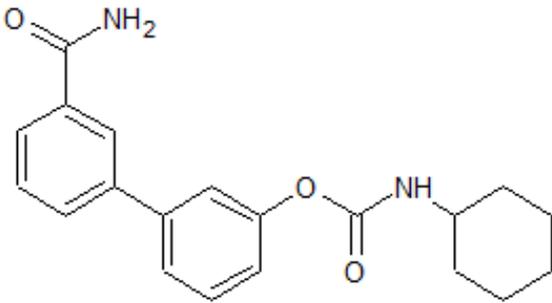
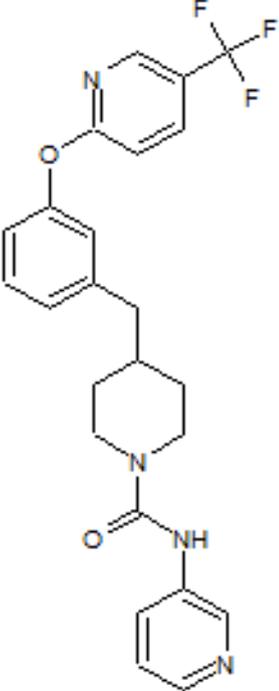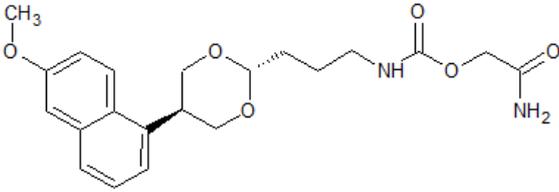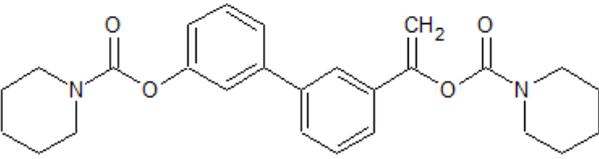## 4. ENDOCANNABINOID MODULATION

4. 1. FAAH

FAAH is a homodimer serine hydrolase and has an integral membrane enzyme structure. It consists of triad: Ser-Ser-Lys, which allows hydrolysis amide bonds. The occurrence of FAAH in the human body remains unclear. Preclinical studies confirm its expression in the brain, lung, spleen, testis, liver and kidney [18]. This enzyme is located on intracellular membranes of postsynaptic cells. Observations of the three-dimensional structure showed the presence of core fold comprised of a β-sheet surrounded by α-helices. Additionally, FAAH contains structure: Ile238-Gly239-Gly240-Ser241 and two channels: acyl chain binding channel A and cytoplasmic access channel A which allow connection to the substrate [19]. This enzyme occurs in two isoforms: FAAH-1 and FAAH-2. Participation in endocannabinoid metabolism is mainly attributed to FAAH-1 [20].

FAAH inhibitors may have a different chemical structure, which may be based on urea, carbamate, miscellaneous, alpha-keto heterocycle, boronic acid, isoxazoline, oxadiazole and indole (Table 2). Moreover, the structures are complex, with hydrophobic domain, active site, membrane access channel that allows access to the substrate and cytosolic port [21, 22].

Table 2. Structures of selected FAAH inhibitors and studies evaluating their potential anxiolytic activity.

Name of compound	Structural formula	Effect of action
URB597 3'-Carbamoyl -[1,1'-biphenyl]-3-yl cyclohexylcarbamate		Anxiolytic effect in the elevated plus maze test [23, 24] Decrease of PTSD-like symptoms in a rat model after chronic treatment [25] Reduction of anxiety induced by 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) [26]

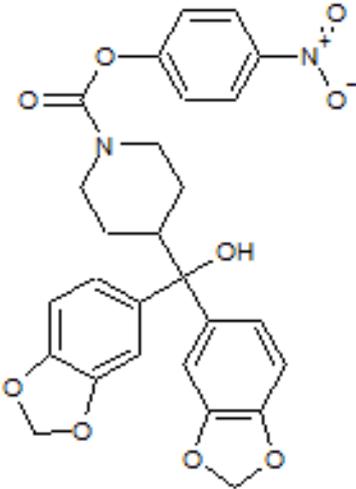|		
PF3845 N-(3-Pyridinyl)-4-(3-{[5-(trifluoromethyl)-2-pyridinyl]oxy}benzyl)-1-piperidinecarboxamide		Anxiolytic effect in the light–dark box test [2] Decrease of anxiety-like behavior caused by 2-AG deficiency after co-administration with THC [2] Rapid anxiolytic effect in anxiety induced by long-term depression (LTD) or in animals chronically exposed to corticosterone [27]
SSR411298 (trans)-2-amino-2-oxoethyl[3-(5-(6-methoxynaphthalen-1-yl)-1,3-dioxan-2-yl)propyl] carbamate		Anti-anxiety effect in the social interaction task [28]
ST4070 1-biphenyl-4-ylethenyl piperidine-1-carboxylate		Anxiolytic effect in elevated plus maze test and light-dark box test [29]

4. 2. MAGL

The enzyme is responsible for 2-AG degradation. It belongs to the cytosolic serine hydrolases with the triad: Ser-His-Asp and contains a specific channel ensuring contact with the substrate and is located at the ends of presynaptic neurons. MAGL expression is observed mostly in the hippocampus, cerebellum and cerebral cortex [15, 20]. Except modulation of endocannabinoids level, MAGL takes part in the degradation of prostaglandin glycerol esters which results in modulation of the immune system [30].
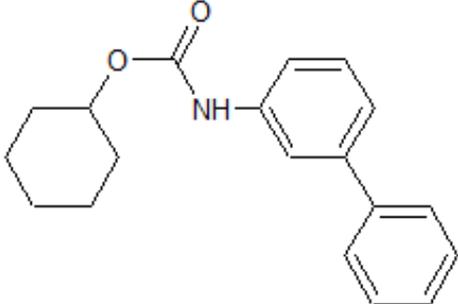
There are three types of MAGL inhibitors. They can act as

- covalent and irreversible
- covalent and reversible
- non-covalent inhibitors [9].

Most MAGL inhibitors are irreversibly combined with the enzyme. This mechanism causes chronic inactivation of MAGL, reduces the sensitivity of the CB1 receptor and impairs synaptic plasticity [31]. Some of these inhibitors have a piperazinyl and triazole structures, others have a triazolopyridine moieties (Table 3). Piperidinyl and piperazinyl carbamates are the most selective MAGL inhibitors, without activity towards FAAH [18]. They have a complex structure that is constantly modified to achieve better selectivity. Structural studies have shown that the addition of an aromatic ring to the enzyme increases the selectivity and reversibility of MAGL inhibitors [23, 24].

Table 3. Structures of selected FAAH inhibitors and studies evaluating their potential anxiolytic activity

Name of compound	Structural formula	Effect of action
JZL184 (4-nitrophenyl) 4-[bis(1,3-benzodioxol-5-yl)-hydroxymethyl]piperidine-1-carboxylate		Prevention of anxiety-like behavior in the novelty-induced hypophagia test [2, 33] Anti-anxiety effect in the light-dark box assay [2] Increased resistance to stress in the novelty-induced hypophagia test [33]

URB602 Cyclohexyl [1,1'-biphenyl] -3-ylcarbamate		Inhibition of panic-like attacks and cardiovascular effects caused by dorsomedial hypothalamus stimulation with NMDA in rats [34]

5. ANXIOLYTIC EFFECT

Endocannabinoids activate not only CB receptors but also serotonin 5-HT_{1A} receptors, transient receptor potential vanilloid 1 (TRPV1) and G protein-coupled receptor 55 (GPR55) located in endothelial cells [6]. The anxiolytic effect associated with increased cannabinoid signaling is dose-dependent and bidirectional: high doses cause anxiogenic, and low doses - anxiolytic effect [5]. The results of behavioral studies showed that inactivation of the FAAH enzyme causes a 10-fold increase in AEA concentration, which stimulates the CB1 receptors, causing anxiolytic effects [6, 35] and inhibits the release of neurotransmitters in two mechanisms.

The first involves the binding to presynaptic CB receptors and inhibition of Ca^{2+} inflow through voltage-dependent Ca^{2+} channels, the second mechanism is associated with the reduction of adenylyl cyclase activity and inhibition of protein kinase A. Both mechanisms lead to a decrease in neurotransmitter release [1,10]. Thus, CB receptor agonists modulate the release of neurotransmitters i.e. acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA), glutamate, d-aspartate and cholecystokinin, involved in the pathomechanism of anxiety [13]. When CB receptors are activated, endocannabinoids are removed from the synaptic cleft and hydrolysed by FAAH and MAGL. Inhibitors of these enzymes prevents degradation of AEA and 2-AG and enable a longer activation time for CB receptors [36]. since endocaabinoids are metabolized not only by FAAH and MAGL, but also by lipoxygenase (LOX) and cyclooxygenase (COX-2), pharmacological modulation of this transmission in anxiety is hindered [20].

Classical pharmacotherapy of anxiety is characterized by delayed therapeutic response. In the initial period, the use of SSRIs provokes transient worsening of symptoms, which is probably associated with compensatory synaptic changes leading to decrease in serotonergic activity.

The most serious consequence of this effect are suicidal thoughts and attempts. Rapid anxiolytic effects of FAAH and MAGL inhibitors makes them an adjunctive therapy in the treatment of anxiety disorder. The use of endocannabinoid modulators could also reduce occurrence of side effects. In addition, they take part in a permanent reduction of anxiety by suppressing the memory of fear [1].

6. SIDE EFFECTS

In addition to therapeutic action of FAAH and MAGL inhibitors, they possess also unwanted side effects. Their occurrence in behavioral studies depends on many factors, including the dose or progress of the disease. Inhibition of FAAH and MAGL occurs not only in the brain but also in the peripheral nervous system. Numerous behavioral studies have shown that FAAH and MAGL inhibitors impairs cognitive performance in a variety of memory assays [2, 38]. In addition, CB1 receptors in limbic forebrain, take part in the regulation of appetite and they can increase weight gain [18]. Moreover, activation of CB1 receptors present in cells Langerhans β, caused by the use of FAAH and MAGL inhibitors, leads to an increase in insulin secretion, which causes hyperinsulinemia and subsequent insulin resistance [6, 39].

On the periphery, endocannabinoids play an important role in the regulation of the digestive system and side effects of endocannabinoid degradation inhibitors include diarrhea or constipation, nausea, somnolence, dizziness and headache [37].

7. CONCLUSIONS

The pharmacological manipulation of endocannabinoid signaling at the level of cannabinoid receptors, transporters or degradative enzymes is a potential strategy for regulating fear and anxiety. Many preclinical tests confirm FAAH and MAGL inhibitors effectiveness. Both types of inhibitors have a different chemical structures that determine their therapeutic effect.

The fact that FAAH and MAGL inhibitors regulate the level of endogenous cannabinoids that are released on demand is a key therapeutic mechanism because it prevents excessive stimulation of CB receptors and, consequently, their desensitization. In addition, such specific inhibition of endocannabinoid degradation at strategic sites limits the occurrence of many potential side effects.

Further modification of FAAH and MAGL inhibitor structures is needed to increase efficacy and reduce side effects. Extending research on these inhibitors could be an important step in the treatment of anxiety disorders. Since recent studies have uncovered involvement of endocannabinoids in other diverse (patho)physiological processes such as pain and depression, therefore inhibitors of endocannabinoids degradation could be used in treating a broad array of complex human diseases. However, due to the limited number of studies available, these compounds are not yet been approved for human use.

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