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Endocannabinoid system and mood disorders: Priming a target for new therapies

Vincenzo Micale [a,b,c,*], Vincenzo Di Marzo [d], Alexandra Sulcova [a], Carsten T. Wotjak [b], Filippo Drago [c]

[a] CEITEC (Central European Institute of Technology) Masaryk University, Brno, Czech Republic
[b] Max Planck Institute of Psychiatry, Research Group "Neuronal Plasticity", Munich, Germany
[c] Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Catania, Italy
[d] Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Naples, Italy

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ABSTRACT

The endocannabinoid system (ECS), comprising two G protein-coupled receptors (the cannabinoid receptors 1 and 2 [CB1 and CB2] for marijuana's psychoactive principle Δ^9-tetrahydrocannabinol [Δ^9-THC]), their endogenous small lipid ligands (namely anandamide [AEA] and 2-arachidonoylglycerol [2-AG], also known as endocannabinoids), and the proteins for endocannabinoid biosynthesis and degradation, has been suggested as a pro-homeostatic and pleiotropic signaling system activated in a time- and tissue-specific way during physiopathological conditions. In the brain activation of this system modulates the release of excitatory and inhibitory neurotransmitters and of cytokines from glial cells. As such, the ECS is strongly involved in neuropsychiatric disorders, particularly in affective disturbances such as anxiety and depression. It has been proposed that synthetic molecules that inhibit endocannabinoid degradation can exploit the selectivity of endocannabinoid action, thus activating cannabinoid receptors only in those tissues where there is perturbed endocannabinoid turnover due to the disorder, and avoiding the potential side effects of direct CB1 and CB2 activation. However, the realization that endocannabinoids, and AEA in particular, also act at other molecular targets, and that these mediators can be deactivated by redundant pathways, has recently led to question the efficacy of such approach, thus opening the way to new multi-target therapeutic strategies, and to the use of non-psychotropic cannabinoids, such as cannabidiol (CBD), which act via several parallel mechanisms, including indirect interactions with the ECS. The state of the art of the possible therapeutic use of endocannabinoid deactivation inhibitors and phytocannabinoids in mood disorders is discussed in this review article.

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Abbreviations: 5-HT, 5-Hydroxytryptamine or serotonin; AA-5-HT, N-arachidonoyl-serotonin; ACEA, arachidonoyl 2′-chloroethylamide; AEA, N-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; AM281, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide; AM404, *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide; AM630, [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone (6-iodopravadoline); AM3506, 5-(4-hydroxyphenyl)pentanesulfonyl fluoride; AM4113, N-piperidin-1-yl-2,4-dichlorophenyl-1H-pyrazole-3-carboxamide; AMY, amygdala; Bl6, C57Bl6; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; CBDV, cannabidivarin; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CDP, chlordiazepoxide; CIT, citalopram; CNR1, human CB1 receptor gene; CNS, central nervous system; CMS, chronic mild stress; CP55940, (-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; CREM, cremophor; dlPAG, dorsolateral periaqueductal gray; DMI, desipramine; DMSO, dimethylsulfoxide; DW, distilled water; DZP, diazepam; EPM, elevated plus-maze; ECS, endocannabinoid system; ETOH, ethanol; FAAH, fatty acid amide hydrolase; FC, fear conditioning task; FLU, fluoxetine; FST, forced swim test; GABA, γ-aminobutyric acid; HPβCD, hydroxypropyl-β-cyclodextrin; HU-210, (6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy- 6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol; I.C.V., intracerebroventricular route of administration; IMI, imipramine; I.P., intraperitoneal route of administration; JWH-015, (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone; JWH-133, (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran; JZL184, 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate; KO, knock-out mice; LD, light-dark avoidance task; LE, long evans; MAGL, monoacylglycerol lipase; MAOI, monoamine oxidase inhibitor; MBB, marble burying behavior; MDZ, midazolam; NADA, N-arachidonoyl-dopamine; NE, noradrenalin; NRI, norepinheprine reuptake inhibitor; NST, non-stressed group; OBX, olfactory bulbectomy; OEA, oleoylethanolamide; PAR, paroxetine; PEA, palmitoylethanolamide; PEG, polyethyleneglycol; PFC, prefrontal cortex; PND, postnatal day; P.O., *per os* route of administration; PPAR, peroxisome proliferator-activated receptor; SAL, saline; S.C., subcutaneous route of administration; SD, sprague dawley; SNRI, serotonin norepinheprine reuptake inhibitor; SPT, sucrose preference test; SSRI, selective serotonin reuptake inhibitor; SR144528, *N*-((1*S*)-endo-1,3,3-trimethyl bicyclo heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide); STR, stressed group; Tw80, tween 80; TCA, tricyclic antidepressant; Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin; TRPV1, transient receptor potential vanilloid 1 channel; TST, tail suspension test; URB597, cyclohexylcarbamic acid 3_-carbamoyl-biphenyl-3-yl ester; vHPC, ventral hippocampus; vmPFC, ventromedial prefrontal cortex; WIN55,212-2, [3H]norepinephrine,(R)-(þ)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanonemesylate; WT, wild type mice.

* Corresponding author at: CEITEC (Central European Institute of Technology) Masaryk University, Kamenice 5/A19, 62500 Brno, Czech Republic. Tel.: +420549494624; fax: +420549492364.
E-mail address: vincenzomicale@inwind.it (V. Micale).

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Contents

1. Introduction

1.1. Current pharmacological approach for the treatment of the major mood disorders

The two major mood disorders such as depression and anxiety are the most prevalent forms of mental illness with 17% lifetime prevalence, resulting in enormous personal suffering, as well as social and economic burden (Lopez & Murray, 1998; Kessler et al., 2005; Wittchen et al., 2011). The major depressive disorder is characterized by episodes of depressed mood lasting for more than 2 weeks often associated with feelings of guilt, low-self esteem and worthlessness and high anxiety. It is also accompanied by additional symptoms including disturbed sleep and appetite, impairment in memory and suicidal thoughts (American Psychiatric Association, 2000). The treatment of depression was revolutionized more than 50 years ago with the discovery—by serendipity—that pharmacological agents such as the tricyclic antidepressants "TCAs" and the monoamine oxidase inhibitors "MAOIs", by enhancing the synaptic levels of monoamines, improved the symptoms of depression, leading to the *monoamine hypothesis of depression* (Schildkraut, 1965). Thus, the introduction of antidepressant drugs had a profound impact on the way depression is viewed: if chemicals can reverse most of depressive symptomatology, then depression itself may be due to chemical abnormalities in the brain. However, due to their toxic and poorly tolerated profile, first generation antidepressants were largely replaced by the selective serotonin reuptake inhibitors (SSRIs), norepinheprine reuptake inhibitors (NRIs) and serotonin norepinheprine reuptake inhibitors (SNRIs) and by atypical antidepressants (i.e. mirtazapine and nefazodone), which show an improved side effects profile but are not more effective than TCAs or MAOIs (Li et al., 2012). Recently, some atypical antipsychotics such as quetiapine, olanzapine or aripiprazole, used either as monotherapy or in combination with sertraline or venlafaxine have also shown efficacy at ameliorating symptoms of bipolar depression and treatment-resistant major depression and received FDA approval for these indications (Kupfer et al., 2012). Since a dysregulation of circadian rhythm has been recognized as a major contributor or a sequel of mood disturbance, agomelatine, a melatonergic agonist and a 5-HT_{2C} antagonist elicited antidepressant activity with a relatively mild side-effect profile, representing a new concept for the treatment of mood disorders (Sansone & Sansone, 2011).

However, the past decade has witnessed a driven focus on the rational discovery of highly selective drugs, acting at innovative non monoaminergic targets such as glutamatergic and GABAergic neurotransmission, neuropetide signalling or neuroendocrine system, which in turn, could affect intracellular signal transduction pathways; but—except for the NMDA receptor antagonist ketamine (Duman & Aghajanian, 2012)—none of these drug has reached the market (Machado-Vieira et al., 2009; Kehne & Cain, 2010; Wong et al., 2010; Engin et al., 2012). Thus, the dominant model of depression is still the monoamine model, which alterations are the primary target for current antidepressant medications. Although today's treatments are generally safe and effective, 30% of depressed patients treated with antidepressants available already on the market are resistant to these drugs. In addition, it is necessary to administer these drugs for weeks or months to see clinical benefit (Connolly & Thase, 2012). Therefore, there is still a great need for faster acting, safer and more effective treatments for depressive disorders.

The anxiety disorders, including panic disorder, social anxiety disorder, generalized anxiety disorder, obsessive–compulsive disorder and post-traumatic stress disorder, share the features of apprehension about future events (associated with symptoms of anxiety) and avoidance behavior (American Psychiatric Association, 2000). With the introduction of chlordiazepoxide as a psychotherapeutic agent in 1960 (Tobin & Lewis, 1960), benzodiazepines, which act to enhance the actions of γ-aminobutyric acid (GABA) neurotransmission, replaced barbiturates and became the mainstay of pharmacotherapy for anxiety disorders. In the late 80s buspirone emerged as the first non-benzodiazepine anxiolytic drug approved for the treatment of generalized anxiety disorder. However, buspirone did not replace the use of benzodiazepines in the clinical management of anxiety, partly because of ongoing concerns about its efficacy (Rickels et al., 1982). An important development in the pharmacotherapy of anxiety disorders was the introduction of antidepressant treatment, which was based on the recognition that a degree of neurobiological commonality exists between depressive and anxiety disorders, as implied by their high degree of co-occurrence (Morilak & Frazer, 2004). Actually, several guidelines argue that SSRIs or SNRIs are first-line pharmacotherapy for a number of anxiety disorders (Baldwin et al., 2012). Although benzodiazepines, SSRIs, and SNRIs are often effective, it is clear there is a need for improvement in the development of rapidly acting, better tolerated medications with a greater and more sustained response.

Therefore, the comorbidity and symptomatic overlap between depressive and anxiety disorders, along with the partial efficacy of actual pharmacological armamentarium, raises the central question to be addressed in this review: Should the pharmacological exploitation of the endocannabinoid system (ECS) be a promising future option to treat the behavioral dimensions which are dysregulated in both depressive and anxiety disorders, and account for the high degree of comorbidity and overlapping symptomatology between these two affective disorders?

1.2. Cannabis and mental illness: clinical evidence on depression and anxiety

Cannabis (or marijuana) is the most frequently abused illicit "recreational" substance in the Western society, its popularity being due to its capacity to alter sensory perception, to induce euphoria and to increase sociability. Although the association between *Cannabis sativa* and psychopathologic conditions has been known for thousands of years before the Christian era, only in the last 50 years the identification of the chemical structure of marijuana components, the cloning of specific cannabinoid receptors and the discovery of the ECS in the brain has triggered an exponential growth of studies to explore its real effects on mental health (Pacher et al., 2006). The *Cannabis* plant contains over 100 terpenophenolic pharmacologically active compounds, known as cannabinoids. Of these, Δ-9-tetrahydrocannabinol (Δ^9-THC), characterized in 1964 (Gaoni & Mechoulam, 1964), was identified as the main psychoactive component of *Cannabis*, and later shown to act as a direct agonist of cannabinoid CB1 and CB2 receptors. Other cannabinoids

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include cannabigerol (CBG), cannabichromene (CBC) and cannabidiol (CBD), which do not seem to cause any Δ^9-THC-like psychoactivity. They exert effects via several mechanism, including modulation of EC tone (Bisogno et al., 2001; Carrier et al., 2006; De Petrocellis et al., 2011), interaction with vanilloid TRPV1 channels (Bisogno et al., 2001) and serotonin 5-HT_{1A} receptors (Russo et al., 2005), and enhancement of adenosine signaling (Magen et al., 2009; Cascio et al., 2010). As recently reviewed, the above mentioned mechanisms could underlie the positive effects induced by CBD treatment in preclinical studies of several disorders (Izzo et al., 2009; Hill et al., 2012b).

In addition to the recreational actions of *Cannabis*, many anecdotal reports from patients attest its acute antidepressant, anxiolytic and stress-relieving effects, which were recently further supported in a controlled clinical study showing that the synthetic Δ^9-THC, dronabinol, facilitated fear extinction (Grinspoon & Bakalar, 1998; Iversen, 2003; Ashton et al., 2005; Rabinak et al., 2013). On the other hand, a biphasic effect of cannabinoids in humans, which has already been shown in rodents (Sulcova et al., 1998), is supported by several data since high doses or rapid administration of Δ^9-THC as well as chronic *Cannabis* use are associated with transient psychotic syndrome, panicogenisis and bipolar disorders, which could be due to the Δ^9-THC capacity to modulate several neurotransmitter systems (Piomelli, 2003). It is not to be excluded that other factors such as the dose, route of administration, baseline emotional states, personality, environment and the setting during which the drug is used, could be involved in Δ^9-THC effects on mood. Because of such bidirectional effects of cannabinoids in humans, recent research has primarily focused on the role of the ECS in the pathogenesis and treatment of stress-related disorders.

Although preclinical evidence support a dysfunction of EC signaling as a molecular underpinning of psychiatric disorders (Parolaro et al., 2010), to date there are few direct investigations into EC activity in patients with mood disorders. While Hungund et al. (2004) reported a significant increase of cannabinoid CB1 receptor density in the prefrontal cortex (PFC) of depressed suicide victims, possibly suggesting a hyperfunction of the ECS in this population, a down regulation of the ECS activity was suggested by Koethe et al. (2007) and Hill et al. (2008), showing a decreased CB1 receptor density in grey matter glial cells and lower EC serum concentration in patients with major depression and anxiety, respectively. However, significantly enhanced serum anandamide (AEA) level in patients suffering of minor depression was also reported (Hill et al., 2008). Furthermore, in two recent clinical studies, a positive correlation was found both between high blood pressure and serum contents of ECs in depressed females (Ho et al., 2012) and among intense exercise, AEA and BDNF levels (Heyman et al., 2012), suggesting that an interrelationship among ECs, depression and cardiovascular risk factors in women and an increase in peripheral BDNF levels could be a mechanism by which AEA intervenes in the neuroplastic and antidepressant effects of exercise. Thus, considering the recent preclinical evidence relating the effects of enhanced EC signaling to promote neurogenesis, it is not to exclude that its activation exerts antidepressant properties through mechanisms that resemble the ones triggered by conventional antidepressants on synaptic plasticity (Duman & Monteggia, 2006; Hill et al., 2009). Despite these few clinical data, increasing interest concerning ECS dysfunction in depressive disorders was engendered after that the clinical use of the cannabinoid CB1 antagonist rimonabant against obesity was interrupted. In line with the theory that a decreased CB1 receptor signaling could be involved in depression, rimonabant used in obese populations was withdrawn from the market because of undesiderable psychiatric side effects such as depression, anxiety and suicidal ideation (Moreira et al., 2009).

Although no controlled clinical trials concerning EC signaling in depression are available, opposite changes in EC activity could underlie the different forms of depressive illness. As recently suggested, genetic variations in CB1 receptor function could also facilitate the development of stress related disorders in humans (Lazary et al., 2011). The human CB1 receptor gene (CNR1), which is located at the chromosome 6q14-15, seems to be implicated in a broad spectrum of psychiatric disorders such as schizophrenia, substance abuse disorders and autism spectrum conditions (Levinson et al., 2000; Chen et al., 2008; Chakrabarti & Baron-Cohen, 2011). Regarding depression, while Barrero et al. (2005) showed a significant association between polymorphisms in CNR1 and depression only in Parkinson`s disease patients, recent studies support that genetic variations in CB1 receptor function and in fatty acid amide hydrolase (FAAH), the enzyme responsible for the inactivation of the endocannabinoid AEA, could influence both the development of depressive symptoms and the antidepressant treatment response (Domschke et al., 2008; Juhasz et al., 2009; Monteleone et al., 2010). Despite the fact that several reports suggest that *Cannabis* use could affect state and trait of anxiety, the few studies available assessing specifically the role of polymorphisms in proteins of the ECS in the development anxiety disorders have provided controversial results. Lu et al. (2008) found a significant association between variants in CNR1 gene and post-traumatic stress disorder in ADHD patients with anxiety symptoms, which was not replicated by Juhasz et al. (2009). However, a significant genetic interaction among variants in the CNR1 gene, polymorphism in the serotonin transporter gene 5-HTTLPR, anxiety or stress adaptation has been also found (Lazary et al., 2009; Agrawal et al., 2012). Thus, the identification of individuals with high-risk of psychiatric disorders through genetic testing could be a good strategy for the development of safer drugs.

2. The endocannabinoid system (ECS)

The ECS plays a role in a variety of physiological processes both in the central nervous system (CNS) and in the periphery, where it acts as neuromodulator at inhibitory and excitatory synapses in brain regions involved in emotional or non-emotional behavior, and mediates the effects of the psychoactive constituent of *Cannabis* Δ^9-THC (Isbell et al., 1967). Considerable evidence in almost each of the major therapeutic areas of interest supports the concept that alterations in some component of the ECS are associated with disease. These include pain and inflammation (Di Marzo, 2012b); immunological disorders (Svízenská et al., 2008; Bíró et al., 2009) neurodegenerative (Mazzola et al., 2003; Micale et al., 2007, 2010) and stress-related conditions (Riebe & Wotjak, 2011) obesity, metabolic (Di Marzo et al., 2011; Di Marzo, 2012a) and cardiovascular (Montecucco & Di Marzo, 2012) diseases; cancer (Velasco et al., 2012), gastrointestinal (Izzo & Sharkey, 2010; Di Marzo & Piscitelli, 2011) and hepatic (Silvestri et al., 2011) disorders. However, the exact pathophysiological mechanisms through which the ECS plays these functions are not fully elucidated yet. The ECS is comprised of: (1) the cannabinoid receptors CB1 and CB2 (Howlett et al., 1990; Matsuda et al., 1990; Munro et al., 1993), (2) the CB1 and CB2 endogenous ligands, anandamide (N-arachidonoyl-ethanolamine, AEA) and 2-arachidonylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995), (3) a specific and not yet identified cellular uptake mechanism and (Lovinger, 2007; Marnett, 2009) (4) the enzymes for endocannabinoid biosynthesis: *N* acyl-phosphatidylethanolamine-selective phosphodiesterase or glycerophosphodiesterase E1 and diacylglycerol lipase α or β (Di Marzo & Petrosino, 2007; Liu et al., 2008); or inactivation: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) (Cravatt et al., 1996; Dinh et al., 2002), respectively for AEA and 2-AG. Despite strong evidence supporting that AEA is an endogenous ligand for cannabinoid CB1 receptors in the brain, some of the typical cannabimimetic effects of AEA are still present in cannabinoid CB1 receptor knock-out mice. These effects may be due to AEA capability to act as a full agonist for the transient receptor potential vanilloid 1 (TRPV1) (Starowicz et al., 2007), of which capsaicin, ingredient of hot red pepper, is considered the exogenous ligand and which has signaling mechanisms distinct from CB1 and CB2 receptors. However, the complexity of the ECS is also due to the growing numbers of additional "players" which are currently described as potential members of this signaling system,

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including putative CB1 antagonist peptides like hemopressins, peroxisome proliferator-activated receptor-α (PPAR-α) and γ (PPAR-γ) ligands, such as oleoylethanolamide (OEA) or palmitoylethanolamide (PEA), and N-arachidonoyl-dopamine (NADA), which activates both TRPV1 and CB1 receptors. Although the existence of a third cannabinoid receptor subtype has been also suggested (Begg et al., 2005), to date only CB1 and CB2 receptors are recognized as G protein coupled receptors for endocannabinoids (Pertwee et al., 2010).

The cannabinoid CB1 and CB2 receptors have been identified by molecular cloning and are unambiguously established as mediators of the biological effects induced by cannabinoids, either plant-derived, synthetic, or endogenously produced. They are encoded by two different genes on human chromosomes: 6q14–q15 (CNR1) and 1p36.11 (CNR2). They are 7 transmembrane Gi/o coupled receptors that share 44% protein identity and display different pharmacological profiles and patterns of expression, a dichotomy that provides a unique opportunity to develop pharmaceutical approaches. The CB1 receptors are ubiquitously expressed in the CNS where they are present at high density in the basal ganglia, frontal cortex, hippocampus (HPC) and cerebellum, while with a moderate/low density they are expressed in the periaqueductal gray, amygdala, nucleus accumbens, thalamus and medulla. However, they are also described in non-neuronal cells of the brain such as microglia, oligodendrocytes and astrocytes (Mackie, 2005). Within these cortical areas there are two major neuronal subpopulations expressing the CB1 receptors: the GABAergic interneurons (with high CB1 receptor levels) and glutamatergic neurons (with relatively low CB1 receptor levels) (Marsicano & Lutz, 1999), which represent the two major opposing players regulating the excitation state of the brain, namely GABAergic interneurons being inhibitory and glutamatergic neurons being excitatory. Recent studies have shown that CB1 receptors are also located in neurons of the dorsal raphe (DR) nucleus and in the nucleus coeruleus (LC) which are the major source of serotonin (5-HT) and noradrenalin (NE) in the brain (Häring et al., 2007; Oropeza et al., 2007). Thus, the direct or indirect modulation of monoamine activity or of GABA and glutamate neurons, respectively, could underlie the psychotropic and non-psychotropic effects of CB1 activation. The cannabinoid CB2 receptors, which are also activated by AEA and 2-AG, are mainly distributed in immune tissues and inflammatory cells including spleen, tonsils, thymus, lymphocytes and macrophages, although they are also detected in glial cells, and to a much lesser extent, in neurons of several brain regions such as cerebral cortex, HPC, amygdala, hypothalamus and cerebellum (Van Sickle et al., 2005; Gong et al., 2006). They play an important part in pain and inflammation even though recent data suggest their involvement in emotional and non emotional processes (Marco et al., 2011). The observation that the elements of the EC neuromodulatory system are prevalent throughout the neuroanatomical structures and circuits implicated in emotionality, including the PFC, HPC, amygdala, hypothalamus and forebrain monoaminergic circuits, provides a rationale for the preclinical development of agents targeting the ECS to treat affective diseases.

3. How to assess the potential antidepressant/anxiolytic activity of a drug: are current animal models a reliable tool?

Since current treatments for anxiety and depression are of limited efficacy in a considerable proportion of patients, and are associated with troublesome side-effects that reduce compliance, a better understanding of the pathophysiology of these disorders and the development of novel, improved therapeutic treatments would fill a considerable unmet medical need (Millan, 2009). Due to the enormous cost of clinical trials, pharmaceutical companies make all efforts at testing new chemicals designed to alter the function of a specific target of disease in a predictable and safe manner. Thus, of central importance to this approach is the availability of valid preclinical animal models for the evaluation of the potential efficacy of novel compounds and the further understanding of the neuropathology that underlies the idiopathic disease state of depression (DiMasi et al., 2003). Ideally, an experimental animal model should reflect the human psychiatric disease in terms of face validity (i.e. reproduce the symptoms of depression observed in human), construct validity (the same neurochemical mechanisms in humans as in the animal model) and predictive validity (chronic antidepressant treatment must reverse the phenotype of the animal model) (McKinney & Bunney, 1969). In the case of depression, it is still difficult to envision an animal model which perfectly includes the etiology, the pathophysiology and the symptoms of depression whilst allowing evaluating the responses to treatment. However, there are different models, each with some limitation, but each able to reproduce each single etiological factor or symptom of depression or with some predictive value to identify new treatment agents. For this purpose, the forced swim test (FST) or the tail suspension test (TST) and the chronic mild stress (CMS) or the olfactory bulbectomy (OBX) seem to be a good experimental approach to screen new antidepressants and shape the underlying disease etiology, respectively (Nestler et al., 2002). The most widely used paradigm to assess antidepressant-like behavior is the FST also known as Porsolt`s test (Porsolt et al., 1977). The FST takes advantage of the observation that rodents, following initial-escape orientated movements, rapidly adopt a characteristic immobile posture in an inescapable cylinder filled with water. In this paradigm, immobility is interpreted as a passive stress-coping strategy or depression-like behavior (behavioral despair). The FST has shown its ability to detect a broad spectrum of substances with antidepressant efficacy, as these drugs shift the passive stress-coping towards active coping, which is detected as reduced immobility. Furthermore, the quantity of different movements such as climbing and swimming behavior has predictive value to differentiate between NEergic and 5-HTergic activity. The paradigm is easy to perform and has proven its reliability across laboratories. Some of the most representative potential antidepressants with different mechanisms of action have been submitted to this test (Cryan et al., 2005b). Similar assumptions and interpretations as the FST is the TST (Steru et al., 1985). In this test, mice are suspended by their tails for a defined period of time and their immobility is decreased by a broad spectrum of antidepressants. A major drawback of the TST is that its application is restricted to mice and limited to strains which do not tend to climb their tail, a behavior that would otherwise confuse the interpretations of the results (Cryan et al., 2005a).

A different model is the CMS paradigm, which is based on reduced sweet fluid intake as an index of anhenodia, induced by repeated (at least 2 weeks) exposure to unpredictable stressors (i.e. wet bedding, disruption of dark-light cycle and food or water deprivation) (Willner et al., 1987). This model induces various long-term behavioral and neurochemical alterations resembling some of the dysfunctions observed in depressed patients, which are reversed only by chronic treatment with a broad spectrum of antidepressants. Although the CMS model has been hampered by poor inter-laboratory reliability, it emphasizes the predominant role of stress in the etiological cause of depression. The OBX, a lesion model of depression, results in a disruption of the limbic hypothalamic axis followed by neurochemical (i.e. changes in all major neurotransmitter systems) and behavioral (i.e. hyperactive response in the open field paradigm) alterations, which resemble changes seen in depressed patients and are reversed by antidepressants (Cryan et al., 2002).

Fear and anxiety are defined as the response of a subject to real or potential threats which could impair its homeostasis (so called 'normal' anxiety). When this response is excessive or maladaptive, it is defined as 'pathological' anxiety (American Psychiatric Association, 2000). As described in excellent review articles (Belzung & Griebel, 2001; Cryan & Sweeney, 2011), most of the behavioral methods which evaluate the normal or state anxiety, are grouped into two main subclasses: the unconditioned-based procedures and the conditioned responses tests. The majority of studies using animal models of 'normal' anxiety employ unconditioned-based procedures. Among these, the elevated plus-maze (EPM) has become one of the most popular behavioral

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tests for research on anxiety. It is based on the conflict between two opposing innate motivations: on the one hand the drive to explore a novel environment and, on the other hand, the avoidance of potentially dangerous places such as elevated open space and/or brightly lit compartment (Pellow et al., 1985). Similarly to the EPM, the light/dark box test (LD) is based on the innate aversion of rodents to brightly illuminated areas and on their spontaneous exploratory behavior in response to mild stressors, that is, novel environment and light (Crawley & Goodwin, 1980). Anxiolytic drugs shift the balance between approach and avoidance toward approach responses. A different unconditioned model is the marble burying behavior (MBB) test which is widely used as a model of obsessive–compulsive disorder. In this test, a mouse is placed into a clean cage filled with a level layer of bedding, covered with glass marbles. The marbles are disturbed and become covered as the mouse digs into the bedding. Thus, the number of marbles buried correlates with the frequency of digging bouts. Interestingly, marble burying is decreased by traditional anxiolytics, such as benzodiazepines as well by SSRIs (Albelda & Joel, 2012). The second class of tests is based on Pavlovian conditioning, where the animals associate an a priori neutral stimulus (i.e. cued fear conditioning) or environment (i.e. contextual fear conditioning) with a punishment. On subsequent confrontation with that stimulus, animals show a number of characteristic fear responses such as freezing and potentiated startle, which are diminished following repeated exposure to the fear-eliciting stimuli in a process termed fear extinction (Myers & Davis, 2007). In most of the tests described above, locomotor activity in the open field test must be also monitored to ensure that motor depression rather than emotional behavior is not influencing the responses (Cryan et al., 2002). Although none of the available experimental models is able to model all aspects of depression and anxiety disorders in terms of etiological factors and symptoms, and most likely never will, the paradigms described above have proven extremely useful both in the identification of potential new drugs to treat mood disorders and in the validation of neurobiological concepts. More specifically, they have been extensively used to assess the potential antidepressant- and or anxiolytic-like activity of compounds modulating the ECs signaling in rodents.

4. Effects of pharmacological exploitation of the EC signaling in preclinical studies of mood disturbances

Since the identification of cannabinoid CB1 and CB2 receptors and their endogenous ligands (AEA and 2-AG) a key aspect in the assessment of the function and therapeutic potential of the ECS has been the availability of useful and selective pharmacological tools. These compounds vary from directly acting compounds such as agonists and inverse agonists (Fig. 1A) to agents that enhance indirectly EC signaling (Fig. 1B). In turn, the latter tools, such as AM404, VDM11 or UCM707 may affect the cellular reuptake of ECs, whereas compounds like URB597, AA-5-HT, AM3506 or JZL184, by inhibiting the hydrolytic enzymes FAAH and MAGL increase brain levels of AEA or 2-AG. Since there are additional elements which can be described as potential members of the ECS such as ligands (i.e. noladin, virodhamide), receptors (GPR55, PPARγ, TRPV1) and synthetic or degradative pathways, it is not to exclude their involvement in the mechanism of action of the compounds described above (Di Marzo, 2008).

4.1. Direct activation of CB1, CB2 or TRPV1 receptors in depression and anxiety

Different substances capable to interact directly with CB receptors and showing different efficacies and selectivity (i.e. from non-selective compounds to CB1, CB2 or TRPV1 selective ligands) have been evaluated in several animal models of mood disorders, as summarized in Table 1. Recent studies (El-Alfy et al., 2010; Bambico et al., 2012), have shown that the main pharmacologically active principle of the *Cannabis sativa*, i.e. Δ^9-THC, decreased immobility time in rodents after acute or repeated (5 days) treatment without any change in the locomotor activity, as assessed in the open field test. However, these studies are not consistent

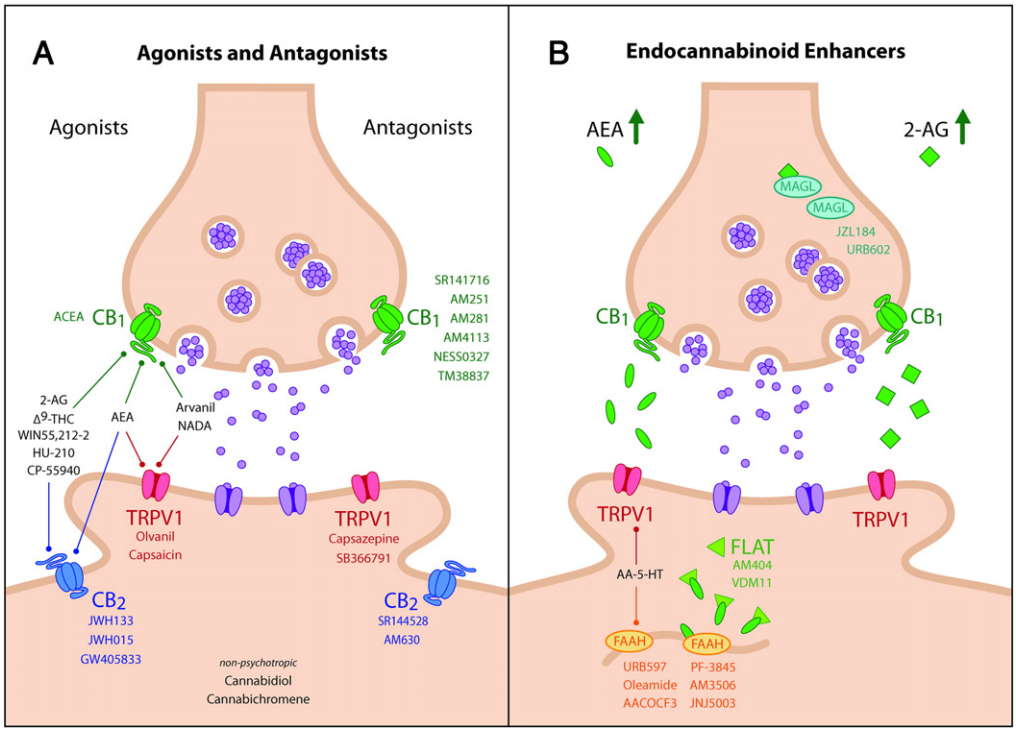

Fig. 1. Schematic illustration of the pharmacological modulation (i.e. agonists, antagonists and endocannabinoid enhancers) of the endocannabinoid system. There is currently no consensus about the preponderance of presynaptic vs. postsynaptic vs. heterosynaptic expression of CB2 and TRPV1. For the matter of clarity we depicted only one of the possible localizations, exemplarily for an excitatory glutamatergic synapse. In contrast, there is a clear compartmentalization of the enzymes implicated in endocannabinoid synthesis and degradation, with endocannabinoid synthesis and AEA degradation occurring in postsynaptic and 2-AG degradation in presynaptic terminals. For details about the different drugs see the main text and Tables 1–4.

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Table 1
Agonists.

Drug	Treatment (days)	Effective dose (range tested)	Route[a]	Vehicle	Animals	Test[a]	Behavioral response	Positive control[a]	References
(a) Selective CB1 receptor agonist									
Arachidonoyl	Acute	0.05 (0.05–5) pmol/rat	dlPAG	0.04% Etoh/sal	Wistar rats	EPM	↓ anxiety	ND	Moreira et al., 2007
2′-chloroethylamide	Acute	5 (0.5–50) pmol/side	plPFC	0.04% Etoh/sal	Wistar rats	EPM	↓ anxiety	ND	Fogaça et al., 2012
(ACEA)	Acute	0.05 (0.01–0.5) pmol/rat	dlPAG	Tocrisolve TM 100	Wistar rats	Panic	↓ panic	ND	Casarotto et al., 2012
(b) Selective CB2 receptor agonists									
JWH133	Acute	1/3/10 (1–10) mg/kg	i.p.	5% Etoh/5% crem/90% sal	Swiss mice	EPM	↓ anxiety	ND	Busquets-Garcia et al., 2011
	Acute	(0.5–2) mg/kg	i.p.	DMSO(1)/Tw80(1)/dw(18)	Swiss mice	LD	↔ anxiety	ND	García-Gutiérrez et al., 2012
	Chronic (7 d, 2/d)	0.5/2 (0.5–2) mg/kg	i.p.	DMSO(1)/Tw80(1)/dw(18)	Swiss mice	EPM/LD	↑ anxiety	ND	García-Gutiérrez et al., 2012
JWH015	Acute	20 (1–20) mg/kg	i.p.	Etoh(1)/emulphor(1)/w(18)	Bl6-DBA/2 mice	LD	↑ anxiety	ND	Onaivi et al., 2008
	Chronic (28 d, 1/d)	20 mg/kg	i.p.	Etoh(1)/emulphor(1)/w(18)	BALBc mice	CMS	↑ sucrose consumption in NST mice	ND	Onaivi et al., 2008
GW405833	Repeated (1 d, 3/d)	30 (10–30) mg/kg	i.p.	25% HPβCD/dw	Wistar rats	FST	↓ immobility in chronic pain model	DMI (20 mg/kg, i.p.)	Hu et al., 2009
(c) Nonselective CB1/CB2 receptor agonists									
Δ^9-THC	Acute	0.3 mg/kg	i.p.	Etoh(1)/crem(1)/dw(18)	CD1 mice	LD	↓ anxiety	ND	Berrendero & Maldonado, 2002
	Acute	1/2.5/10 (0.25–10) mg/kg	i.p.	Etoh(1)/emulphor(1)/sal(18)	ICR mice	EPM	↑ anxiety	ND	Patel & Hillard, 2006
	Acute	2.5 (0.5–2.5) mg/kg	i.p.	Etoh(1)/emulphor(1)/sal(18)	CD rats	EPM/LD	↑ anxiety	ND	Schramm-Sapyta et al., 2007
	Acute	0.075/0.375/0.75/1.5 (0.015–3) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	EPM	↓ anxiety	ND	Rubino et al., 2007
	Acute	5/10 (2.5–25) μg/rat	PFC/vHPC	Etoh(1)/crem(1)/sal(18)	SD rats	EPM	↓ anxiety	ND	Rubino et al., 2008a
	Acute	1 (1–10) μg/rat	AMY	Etoh(1)/crem(1)/sal(18)	SD rats	EPM	↑ anxiety	ND	Rubino et al., 2008a
	Chronic (17 d, 1/d)	10 (0.3–10) mg/kg	i.p.	Etoh(1)/Tw80(1)/sal(18)	Bl6/Jarc mice	EPM/LD	↑ anxiety in LD	ND	Long et al., 2010
	Acute/chronic (7 d, 14 d, 1/d)	2.5 mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	LD	↑ anxiety	ND	O'Brien et al., 2013
	Chronic (11 d, 2/d) (PND35–45)	2.5 (d1–d3) 5 (d4–d7) 10 (d8–d11) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	FST	↑ immobility ♀	ND	Rubino et al., 2008c
	Chronic (11 d, 2/d) (PND35–45)	2.5 (d1–d3) 5 (d4–d7) 10 (d8–d11) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	SPT/EPM	↓ sucrose consumption/↔ anxiety	ND	Rubino et al., 2008c
	Acute	2/6 (1–6) mg/kg	i.p.	1% Tw80/dw	Ddy mice	FST	↑ immobility	CIT (10 mg/kg, i.p.)	Egashira et al., 2008
	Acute	2.5 (1.25–5) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	Swiss-DBA/2 mice	FST/TST	↓ immobility	FLU (40 mg/kg, i.p.), DMI (20–40 mg/kg, i.p.)	El-Alfy et al., 2010
	Chronic (5 d, 1/d)	1 mg/kg	i.p.	5% Tw80/5% PEG/sal	SD rats	FST	↓ immobility	CIT (10 mg/kg, i.p.)	Bambico et al., 2012
	Chronic (21 d, 1/2d)	2 mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	LH rats	OBX	↓ locomotor activity in OBX rats	ND	Elbatsh et al., 2012

Anandamide (AEA)	Acute	5 (0.5–50) pmol/rat	dlPAG	Tocrisolve TM 100	Wistar rats	EPM	↓ anxiety	ND	Moreira et al., 2007
	Acute	5 pmol/side	vmPFC	Tocrisolve TM 100	Wistar rats	FC	↓ freezing	ND	Lisboa et al., 2010
	Repeated (4 d, 1/d)	10 mg/kg	i.p.	DMSO/dw	ICR mice	FST/TST/ CMS	↔ Immobility/↑ sucrose consumption in STR mice	CLM (10 mg/kg, i.p.)	Hayase, 2011a
	Acute	1–5–10–20 (0.1–20) μg/mouse	i.c.v.	Tocrisolve TM 100	Swiss mice	FST/MBB	↓ immobility/↓ anxiety	FLU (5–20 mg/kg, i.p.)	Umathe et al., 2011
	Acute	0.1/10 (0.1–10) μg/rat	PFC	Etoh(1)/crem(1)/sal(18)	SD rats	EPM	↓ anxiety (0.1 μg)/↑ anxiety (10 μg)	MDZ (5 μg/PFC)	Rubino et al., 2008b
2-Arachidonoylglycerol (2-AG)	Acute	(0.1–10) μg/rat	PFC	DMSO(2)/Tw80(1)/dw(7)	SD rat	EPM	↔ anxiety	MDZ (5 μg/PFC)	Rubino et al., 2008b
WIN55,212-2	Repeated (1 d, 3/d)	01./0.2 (0.05–2) mg/kg	i.p.	5% Tw80/5% PEG/sal	SD rats	FST	↓ immobility	CIT (5 mg/kg, i.p.), DMI (10 mg/kg, i.p.)	Bambico et al., 2007
	Chronic (20 d, 1/d) (PND30–50)	0.2/1 (0.2–1) mg/kg	i.p.	5% Tw80/5% PEG/sal	SD rats	FST/SPT/ EPM	↑ immobility (0.2 mg)/↓ sucrose consumption (1 mg)/↔ anxiety	DMI (10 mg/kg, i.p.), DZP (2 mg/kg, i.p.)	Bambico et al., 2010a
HU-210	Acute	0.1 mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(8)	Wistar rats	FC	↓ freezing	ND	Maćkowiak et al., 2009
	Repeated (1 d, 3/d)	5/25 (5–25) μg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	LE rats	FST	↓ immobility	DMI (10 mg/kg, i.p.)	Hill & Gorzalka, 2005
	Chronic (10 d, 2/d)	100 μg/kg	i.p.	DMSO	LE rats	FST	↓ immobility	ND	Jiang et al., 2005
	Repeated (1 d, 3/d)	1/2.5 (1–2.5) μg/side	dHPC	DMSO	SD rats	FST	↓ immobility	ND	McLaughlin et al., 2007
	Chronic (10 d, 1/d)	0.1 mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	SD rats	FST	↓ immobility	DMI (10 mg/kg, i.p.)	Morrish et al., 2009
CP-55940	Acute	0.1 (0.03–0.3) mg/kg	i.p.	15% HPβCD	Wistar rats	FST	↓ immobility	ND	Adamczyk et al., 2008
	Acute	1/50 (1–50) μg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	Bl6N mice	EPM	↓ (1 μg)/↑ (50 μg) anxiety	ND	Rey et al., 2012
(d) Selective TRPV1 agonists									
Olvanil	Acute	0.1/0.2/2/5 (0.1–5) mg/kg	i.p.	Sesame oil	SD rats	FST/EPM	↑ immobility/↑ anxiety	ND	Kasckow et al., 2004
	Acute	1/2.5 (0.1–2.5) mg/kg	i.p.	DMSO/dw	ICR mice	FST/TST	↓ immobility in STR mice	ND	Hayase, 2011b
Capsaicin	Acute	0.1/1/2.5 (0.1–2.5) mg/kg	i.p.	DMSO/dw	ICR mice	FST/TST	↓ immobility in STR mice	ND	Hayase, 2011b
	Acute	200/300/400 (10–400) μg/ mouse	i.c.v.	Tw80(1)/DMSO(2)/sal(7)	Swiss mice	FST/TST	↓ immobility	FLU(2.5–10 μg/i.c.v.)	Manna & Umathe, 2012
(e) Nonselective TRPV1/CB1 agonists									
Arvanil	Acute	0.1/1/2.5 (0.1–2.5) mg/kg	i.p.	DMSO/dw	ICR mice	FST/TST	↓ immobility in STR mice	ND	Hayase, 2011b
N-arachidonyl dopamine (NADA)	Acute	(0.1–10) mg/kg	i.p.	DMSO/dw	ICR mice	FST/TST	↔ immobility	ND	Hayase, 2011b

[a] AMY—amygdala, CIT—citalopram, CLM—clomipramine, CMS—chronic mild stress, dHPC—dorsal hippocampus, dlPAG—dorsolateral periaqueductal grey, DMI—desipramine, DZP—diazepam, EPM—elevated plus maze, FC—fear conditioning, FLU—fluoxetine, FST—forced swim test, i.c.v.—intracerebroventricular, i.p.—intraperitoneal, LD—light-dark avoidance task, MBB—marble burying behaviour, MDZ—midazolam, NST—non-stressed group, OBX—olfactory bulbectomy, PFC—prefrontal cortex, plPFC—prelimbic medial prefrontal cortex,SPT—sucrose preference test; STR—stressed group; TST—tail suspension test, vHPC—ventral hippocampus, vmPFC—ventromedial prefrontal cortex.

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with those described by Egashira et al. (2008) showing an increased immobility time in mice after acute Δ^9-THC treatment. Discrepancies among these studies could be due to the species (rats vs mice) or strain (swiss vs ddy mice) differences in response to the treatment, as well as to a different experimental procedure (standard FST for mice or time of injection) and basal stress related conditions. Interestingly, chronic exposure to Δ^9-THC or WIN55,212-2 in adolescence induced a depressive-like phenotype in adulthood (Rubino et al., 2008c, 2009; Bambico et al., 2010a; Realini et al., 2011). Therefore, these data further support the concept that adolescence is a critical period in which protracted direct CB1 activation by affecting the monoaminergic system could influence mood control (Rubino et al., 2012). To confirm the antidepressant-like effects of CB1 agonists, the endogenous cannabinoid AEA (Hayase, 2011a; Umathe et al., 2011) and the synthetic nonspecific cannabinoid CB1/CB2 receptor agonists WIN55,212-2 (Bambico et al., 2007), HU-210 (Hill & Gorzalka, 2005; Jiang et al., 2005; McLaughlin et al., 2007; Morrish et al., 2009) and CP55,940 (Adamczyk et al., 2008) or the selective CB1 agonist arachidonoyl 2′-chloroethylamide (ACEA) (Rutkowska & Jachimczuk, 2004) improved the behavioral responses of rodents through a CB1- and 5-HTergic or NEergic-mediated mechanisms. Evidence also suggests that cannabinoids, by modulating the physiological and behavioral response to stressful conditions, elicit anxiolityc-like responses (Riebe & Wotjak, 2011). Although, the effects of the cannabinoid agonists are more complex in animal model of anxiety than in those of depression as described above, a general conclusion which could be derived from the contradictory literature is that high and low doses of cannabinoid agonists often cause anxiogenic and anxiolytic effects in experimental models as well as in humans, respectively (Moreira & Wotjak, 2010). More specifically, in some marijuana smokers it has been observed that heavy *Cannabis* use could induce anxiety, panic attacks or psychotic-like states, effects which have mimicked in models predictive of anxiolytic-like activity (Crippa et al., 2009; Casadio et al., 2011). In the EPM, low doses of Δ^9-THC, administered systemically increased the time spent on open arms, an index of anxiolytic-like effects, in rats through a CB1 mediated mechanism (Rubino et al., 2007) but not in mice, where they instead produce a dose dependent reduction in open arm exploration (Patel & Hillard, 2006); thus species-specific effects for Δ^9-THC response might occur. In turn, as suggested by Haller et al. (2007), these differences could be due to the different responsiveness of GABAergic and glutamatergic neurons to CB1 activation in rats and mice, as well as to a difference in the expression, distribution and functional characterization of CB1 receptors. At higher doses, Δ^9-THC induced in rodents anxiogenic-like responses (Patel & Hillard, 2006; Rubino et al., 2007; Schramm-Sapyta et al., 2007; Long et al., 2010; O'Brien et al., 2013). However, in the LD box, a clear CB1-mediated anxiolytic response was found in mice after systemic administration of a low dose of Δ^9-THC, suggesting that the observed effects could also depend on the specific behavioral test used (Berrendero & Maldonado, 2002). Interestingly, the effects of CB1 receptor stimulation induced by Δ^9-THC seem to be related to the brain region involved. While low dose of Δ^9-THC injection in the PFC or HPC elicited anxiolytic effects, at the level of the basolateral amygdala (BLA) it produced anxiogenic response (Rubino et al., 2008a). In line with data described above, the systemic or local (i.e. dorsolateral periaqueductal gray [dlPAG]) treatment with the synthetic non selective CB1/CB2 agonists WIN55,212-2, (Pamplona et al., 2006; Naderi et al., 2008; Campos & Guimarães, 2009; Klugmann et al., 2011) CP55,940 (Patel & Hillard, 2006), HU-210 (Maćkowiak et al., 2009), the endogenous cannabinoid AEA (Lisboa et al., 2010) or the selective CB1 agonist ACEA (Moreira et al., 2007; Fogaça et al., 2012) elicited at lower doses anxiolytic CB1-mediated responses in conditioned (i.e. decreased freezing response) and unconditioned (increased exploration of open arms) behavioral tasks, while, again, higher doses tended to induce anxiogenic-like responses. The reason for these dose dependent patterns is not fully understood and different hypotheses have been put forward. First, the biphasic effect could be due to a different receptor sensitive to some of these compounds, a possibility supported by Rubino et al. (2008b) who showed, with increasing doses of AEA injected into the PFC, a shift from CB1- to TRPV1-mediated actions of the endocannabinoid, thus leading to opposite anxiety responses (i.e.: anxiolityic CB1 mediated effect vs. anxiogenic TRPV1 mediated effects induced by low and high dose, respectively). This finding opens a complex scenario where CB1 receptor-dependent processes are not the only mechanisms for EC-modulation of anxious-like behaviors, but also the TRPV1 channel might become involved (Moreira et al., 2012). In the CNS, TRPV1 is a calcium-permeable cation channel widely expressed in brain regions (i.e. basal ganglia, nucleus accumbens, HPC, cortex) and involved in synaptic plasticity responses to ECs, which in turn could be related to several mental disorders (Cristino et al., 2006; Starowicz et al., 2007, 2008). However, controversial data have been reported following TRPV1 activation in experimental models of mood disorders. While TRPV1 agonists olvanil and capsaicin elicited antidepressant-like effects (Kasckow et al., 2004; Hayase, 2011b; Manna & Umathe, 2012); by contrast, in a social activity paradigm capsaicin induced anxiogenic response (Manna & Umathe, 2011). These discrepancies may be attributed to interspecies differences in TRPV1 ligand affinity or CB1 and TRPV1 receptor distribution, as well as to different experimental conditions, strain and species. The recent development of cell type specific genetic deletion of CB1 receptors has provided a new tool to better understand cannabinoid action, and assess the different role of the neuronal subpopulations of CB1-expressing neurons, such as GABAergic, glutamatergic and dopamine D1 terminals, in the control of emotional behavior (Monory et al., 2007; Jacob et al., 2009; Terzian et al., 2011; Jacob et al., 2012; Metna-Laurent et al., 2012). In a recent study, Rey et al. (2012) showed that the CB1 receptors on GABAergic vs glutamatergic terminals are required for the anxiogenic- vs. anxiolytic-like effects induced by high vs. low doses of the CB1 agonist CP55,940. These findings might open the way to new anxiolytic cannabinoid drugs which specifically target CB1 receptors on glutamatergic terminals.

Despite the fact that cannabinoid CB2 receptors were initially identified in the rat spleen and leukocyte subpopulation in humans (Munro et al., 1993; Galiègue et al., 1995), and in the brain or spinal cord under pathological conditions such as Alzheimer`s disease, multiple sclerosis or amyotrophic lateral sclerosis, respectively (Benito et al., 2003; Yiangou et al., 2006), they were recently detected also in healthy animals in different brain areas such as HPC, amygdala and cerebral cortex and to be related to stress responses, anxiety or depression (Van Sickle et al., 2005). Only recently the role of CB2 receptors in emotional control has been investigated, with results that are still controversial (Marco et al., 2011). While the CB2 agonist JWH015 induced both anxiogenic effects in mice tested in the LD and no effect in the CMS paradigm (Onaivi et al., 2008), the other CB2 agonist, JWH133, on the one hand induced no effect in the LD after acute treatment, and on the other hand it elicited an anxiogenic response after chronic treatment, which was accompanied by molecular changes in different key targets involved in emotional behavior such as $GABA_A\alpha_2$ and $GABA_A\gamma_2$ receptor subunits (García-Gutiérrez et al., 2012). However, the CB2 selective agonist GW405833 was able to reverse the immobility time in the FST of rats subjected to a model of neuropathic pain (Hu et al., 2009), thus underlying that several factors such as the type of drug and doses used, strain or secondary motor alterations could interfere with the interpretation of these behavioral effects. Although previous reports have mostly focused on the analgesic effects elicited by CB2 agonists, which could be due to CB1 receptor involvement as well, the development of selective CB2 drugs as therapeutic tools is attractive in so far, as they are avoid of psychoactive effect (Riether, 2012).

4.2. Pharmacological inactivation of the EC signaling in depression and anxiety

In the last decades, some of the most promising molecules in pharmacological research were selective antagonists/inverse agonists of the CB1 receptors, due to their potential therapeutic effects in obesity

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and addictive disorders. The first such compound was rimonabant (Rinaldi-Carmona et al., 1994), which was introduced into clinical practice as an antiobesity agent in several countries (RIO studies). Due to the higher incidence in treated patients as compared to placebo controls of psychiatric side effects such as mood symptoms, anxiety and suicidal tendencies, rimonabant was eventually withdrawn from the market (Van Gaal et al., 2005). These events affected the entire industrial development of CB1 antagonists, which was interrupted because of psychiatric side effects in clinical trials (Addy et al., 2008; Kipnes et al., 2010). However, possible solutions for the safe use of CB1 antagonists for the treatment of metabolic syndrome could be to determine which patients are at high risk of psychiatric side effects through detailed phenotypic assessments and genetic testing (Lazary et al., 2011) or the use of CB1 receptor inverse agonists (i.e. JD5037) or antagonists acting specifically in the periphery (Ward & Raffa, 2011; Tam et al., 2012). In line with this strategy, we recently found that the peripheral CB1 receptor antagonist TM38837, which already shown marked weight reduction in preclinical studies (Kirilly et al., 2012) has induced a sustained fear response after systemic treatment only at the doses magnitudes higher than rimonabant (Micale et al., 2011). Apart from their counteraction of a possible endogenous anxiolytic and anti-depressant role of endocannabinoids biosynthesized on demand during stressful conditions in brain areas controlling mood, the anxiogenic-like properties of rimonabant and its analogues such as AM251 may be due also to their activity as inverse agonists at constitutively active conformations of CB1 receptors in these areas, as suggested in some preclinical studies (Lafenêtre et al., 2007), which was recently further supported (Thiemann et al., 2009; Plendl & Wotjak, 2010; Kamprath et al., 2011; Dono & Currie, 2012; Dubreucq et al., 2012; Kupferschmidt et al., 2012; O'Brien et al., 2013) (Table 2). Therefore, an alternative approach to avoid the development of psychological side effects could be the use of compounds with less intrinsic biological activity. Along this line, Sink et al. (2008, 2010a,b) and Meye et al. (2012) reported that the neutral CB1 antagonists AM4113 and NESS0327 did not induce any effects either in conditioned or in unconditioned animal models of anxiety. Although more studies are needed, these results rule out against the existence of the above mentioned tone of on demand produced endocannabinoids (if such tone existed an inverse agonist should have produced results similar to a neutral antagonist) and suggest that drugs like AM4113 or NESS0327 may produce a more favorable clinical psychiatric profile with fewer anxiety-related side effects as compared to CB1 inverse agonist activity (Moreira & Crippa, 2009). In line with the pharmacological CB1 inactivation, the genetic blockade of CB1 signaling also leads to an anxiogenic response, as shown by the phenotype of mice with a complete (Haller et al., 2002; Marsicano et al., 2002) or specific deletion of the CB1 receptors in some neuronal subpopulations (Jacob et al., 2009; Kamprath et al., 2009; Terzian et al., 2011; Jacob et al., 2012; Metna-Laurent et al., 2012; Rey et al., 2012), which also show an impaired behavioral performance in the FST (Steiner et al., 2008b,2008c). However, it must be emphasized that few preclinical studies have also reported a paradoxical antidepressant-like activity of rimonabant in rodents (Tzavara et al., 2003; Griebel et al., 2005; Steiner et al., 2008a; Lee et al., 2009; Elbatsh et al., 2012), and that CB1 inverse agonists have been so far shown to produce psychiatric effects only in obese populations. Therefore, it is also possible that these compounds might produce different effects depending on the underlying affective condition of the tested animals and human subjects. In summary, the data, which are summarized in Table 2, provide evidence for a potential role of the inhibition of CB1 signaling in the development of mood disorders even though the screening of individuals with high risk of psychiatric adverse events through genetic testing or the use of neutral CB1 antagonists or CB1 antagonists with limited penetration through the blood-brain barrier should decrease the psychiatric side effects of CB1 blockade.

As previously described for CB2 activation, a controversial picture concerning the effects of CB2 antagonists was also described. While Onaivi et al. (2008) found a partial anxiogenic- but any antidepressant-like effect of the CB2 antagonists SR144528 and AM630, a recent study of García-Gutiérrez et al. (2012) reported that AM630 induces anxiogenic vs. anxiolytic activity in mice after acute vs. chronic treatment. A certain lack of antagonist specificity, in particular at higher doses, hampers the unequivocal interpretation of these findings. Although mutant mice, which lack expression of CB2 receptors, have been generated to circumvent the discrepancies of data obtained with pharmacological CB2 inactivation, these animals presented more pronounced anxiogenic- and depressive-like phenotype, thus not confirming the concept that prolonged inhibition of CB2 signaling could be of potential therapeutic importance (Ortega-Alvaro et al., 2011). The discrepant findings concerning CB2 receptor ligands are usually attributed to difference in receptor drug affinity, dosage, treatment duration, experimental conditions, strain and species. On the other side, the different effects between pharmacological and genetic inhibition of CB2 signaling could be due to compensative mechanisms which could develop in mutant mice.

Due to their co-localization with CB1 receptors in several brain regions (Cristino et al., 2006), the TRPV1 channel could play the role as "the other side of one coin" in the regulation of anxiety (Moreira et al., 2012). To support this hypothesis, Rubino et al., 2008b showed that the anxiogenic or anxiolytic dose of methanandamide were counteracted by a TRPV1 or CB1 antagonist, respectively, an observation recently confirmed by Casarotto et al. (2012). In addition, further evidence regarding TRPV1 involvement in anxiety-like behavior came from the analysis of pharmacological or genetic blockade of these channels. In the study of Marsch et al. (2007), TRPV1 knock-out mice showed a reduced anxiety phenotype in model of unconditioned or conditioned anxiety, which was reproduced by TRPV1 antagonist treatment (Santos et al., 2008; Aguiar et al., 2009; Micale et al., 2009a; Terzian et al., 2009). Recent evidence suggests that the anxiolytic effect of diazepam, which is usually employed in experimental models as positive control to compare the effects the drugs under investigation in terms of potency and efficacy, may be in part dependent on changes in both the endocannabinoid and endovanilloid systems (Manna & Umathe, 2011). These findings open new perspectives to prevent the risks associated with the long-term use of benzodiazepines. In addition they confirm the ECS activation, together with the GABAergic system, is one of mechanisms underlying the anxiolytic properties of diazepam (Naderi et al., 2008; Micale et al., 2009a,2009b). Though increasing evidence suggest a role of TRPV1 in anxiety, a similar function in depression is still ambiguous and only few recent studies suggest that genetic (You et al., 2012) or pharmacological (Manna & Umathe, 2012) inactivation of TRPV1 signaling may elicit antidepressant-like behaviors, through the involvement of 5-HTergic neurotransmission.

4.3. Facilitation of the endogenous cannabinoid signaling via ECs enhancers: FAAH inhibitors, MAGL inhibitors or EC reuptake blockers

Although drugs direct stimulating cannabinoid receptors showed promise for the treatment of mood disorders, they also elicited significant side effects, which preclude their clinical use (Moreira et al., 2009). Thus, the pharmaceutical strategies to minimize the psychotropic side effects of these compounds have gradually shifted the interest towards alternative approaches such as amplifying the effects of AEA and 2-AG, by preventing their deactivation by FAAH and MAGL or by blocking their cellular reuptake (Fig.1B). CB1, FAAH and MAGL are not equally distributed in the brain; thus, treatment with EC breakdown blockers could modulate CB1 function in select brain areas where FAAH and MAGL are expressed, unlike the direct CB1 agonists which affect synapses wherever the receptors are expressed. On the other hand, if endocannabinoids are produced and inactivated on demand during stressful condition selectively in brain areas and circuits that control mood, inhibiting their inactivation would only indirectly activate CB1 receptors in those areas (Petrosino & Di Marzo, 2010). Accordingly, the treatment with

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Table 2
Antagonists.

Drug	Treatment (days)	Effective dose (range tested)	Route[a]	Vehicle	Animals	Test[a]	Behavioral response	Positive control[a]	References
(a) Selective CB1 antagonists									
Rimonabant (SR141716)	Acute	10 (1–10) mg/kg	p.o.	0.1% Tw80/sal	SD rats	EPM	↓ anxiety	DZP (3 mg/kg, i.p.)	Griebel et al., 2005
	Acute	3 (1–3) mg/kg	i.p.	2% Etoh/sal	CD1 mice	EPM	↑ anxiety	ND	Thiemann et al., 2009
	Acute/chronic (7 d, 14 d, 1/d)	2.5 mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	LD	↑ anxiety	ND	O'Brien et al., 2013
	Acute	3 mg/kg	s.c.	2.5% DMSO/Tw80/sal	Bl6N mice	FC	↑ freezing	ND	Plendl & Wotjak, 2010
	Acute	3 mg/kg	s.c.	2.5% DMSO/Tw80/sal	Bl6N mice	FC	↑ freezing	ND	Kamprath et al., 2006
	Acute	3 mg/kg	s.c.	2.5% DMSO/Tw80/sal	Bl6N, CRHR1-KO, CRHR2-KO mice	FC	↑ freezing	ND	Kamprath et al., 2009
	Chronic (7 d, 1/d)	3 mg/kg	s.c.	1.25% DMSO/Tw80/sal	Bl6N mice	FC	↑ freezing in STR mice	ND	Dubreucq et al., 2012
	Chronic (35 d, 1/d)	3–10 mg/kg	p.o.	0.1% Tw80/sal	Wistar rats-Balb/c mice	FST/CMS	↓ immobility/↓ anxiety	FLU (30 mg/kg, p.o.)	Griebel et al., 2005
	Acute	3 (0.3–3) mg/kg	i.p.	2% DMSO/2% crem/sal	Swiss mice	FST	↓ immobility	ND	Tzavara et al., 2003
	Repeated (2 d/3)	10 mg/kg	i.p.	2.5% DMSO/Tw80/sal	Bl6N mice	FST	↓ immobility	DMI (20 mg/kg, i.p.)	Steiner et al., 2008a
	Chronic (10 d, 1/d)	10 mg/kg	i.p.	2.5% DMSO/Tw80/sal	Bl6N mice	FST	↓ immobility	ND	Steiner et al., 2008a
	Chronic (14 d, 1/d)	10 (1–10) mg/kg	p.o.	0.5% methylcellulose/sal	ICR mice	FST	↓ immobility (1d)/↔ (14d)	IMI (15 mg/kg, p.o.)	Lee et al., 2009
	Chronic (21 d, 1/2d)	5 mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	LH rats	OBX	↓ locomotor activity in OBX rats	ND	Elbatsh et al., 2012
AM251	Acute	2/4/8 (2–8) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	SD rats	EPM	↑ anxiety	FG-7142 (10–20 mg/kg, i.p.)	Sink et al., 2010b
	Acute	4/8 (2–8) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	SD rats	FC	↑ freezing	ND	Sink et al., 2010a
	Acute	1 μg (1 ng-1 μg)/side	BLA/CeA	6.66% DMSO/6.66% Tw80/sal	Bl6N mice	FC	↑ freezing	ND	Kamprath et al., 2011
	Acute	200 (10–200) μg/rat	i.c.v.	DMSO	LE rats	EPM	↑ anxiety	ND	Kupferschmidt et al., 2012
	Acute	3 (0.03–3) mg/kg	i.p.	20% DMSO/sal	SD rats	EPM	↑ anxiety	ND	Dono & Currie, 2012
	Acute	2.5/25 (0.25–25) pmol/side	BLA	20% DMSO/sal	SD rats	EPM	↑ anxiety	ND	Dono & Currie, 2012
AM281	Acute	2.5 mg/kg; 0.05 μg/mouse	i.p./HPC	DMSO	Bl6s mice	FC	↑ freezing	ND	Lin et al., 2011
AM4113	Acute	(3–12) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	SD rats	EPM	↔	FG-7142 (10–20 mg/kg, i.p.)	Sink et al., 2010b
	Acute	(3–12) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	SD rats	FC	↔	ND	Sink et al., 2010a
NESS0327	Acute	0.1 mg/kg	i.p.	1% DMSO/4% PEG/5% Tw80	Wistar rats	EPM	↔	Rimonabant (1 mg/kg, i.p.)	Meye et al., 2012
TM38837	Repeated (3 d, 1/d)	100 (10–100) mg/kg	p.o.	0.1% Tw80/1% HPMC/sal	Bl6N mice	FC	↑ freezing	Rimonabant (10 mg/kg, p.o.)	Micale et al., 2011
(b) Selective CB2 antagonists									
SR144528	Acute	20 (1–20) mg/kg	i.p.	Etoh(1)/emulphur(1)/w(18)	DBA/2 mice	LD	↑ anxiety ♂	ND	Onaivi et al., 2008
AM630	Chronic (28 d, 1/d)	(1–3) mg/kg	i.p.	Etoh(1)/emulphur(1)/w(18)	Balb/c mice	CMS	↔ sucrose consumption	ND	Onaivi et al., 2008
	Acute	2/3 (1–3) mg/kg	i.p.	DMSO(1)/Tw80(1)/dw(18)	Swiss mice	LD	↑ anxiety	ND	García-Gutiérrez et al., 2012
	Chronic (7 d, 2/d)	1/2/3 (1–3) mg/kg	i.p.	DMSO(1)/Tw80(1)/dw(18)	Swiss mice	EPM/LD	↓ anxiety	ND	García-Gutiérrez et al., 2012
(c) Selective TRPV1 antagonists									
Capsazepine	Acute	2 (0.2–2) nmol/side	vHPC	10% DMSO/PBS	Wistar rats	EPM	↓ anxiety	ND	Santos et al., 2008
	Acute	1/10 (1–60) nmol/side	mPFC	DMSO	Wistar rats	EPM	↓ anxiety	ND	Aguiar et al., 2009
	Acute	60 (10–60) nmol/rat	dlPAG	DMSO	Wistar rats	EPM	↓ anxiety	ND	Terzian et al., 2009
	Acute	(1–10) nmol/side	BLA	25% DMSO/sw	SD rats	EPM	↔	ND	John & Currie, 2012
	Acute	100/200 (1–200) μg/mouse	i.c.v.	DMSO(2)/Tw80(1)/sal(7)	Swiss mice	FST/TST	↓ immobility	FLU (2.5–10 μg, i.c.v.)	Manna & Umathe, 2012
SB366791	Acute	1 (0.1–2.5) mg/kg	i.p.	10% DMSO/sal	Bl6J mice	EPM	↓ anxiety	DZP (1 mg/kg, i.p.)	Micale et al., 2009°
	Acute	10 nmol/rat	dlPAG	DMSO	Wistar rats	Panic	↓ panic	ND	Casarotto et al., 2012

[a] BLA—basolateral amygdala, CeA—central amygdala, CMS—chronic mild stress, dlPAG—dorsolateral periaqueductal grey, DMI—desipramine, DZP—diazepam, EPM—elevated plus maze, FC—fear conditioning, FLU—fluoxetine, FST—forced swim test, HPC—hippocampus, IMI—imipramine, i.c.v.—intracerebroventricular, i.p.—intraperitoneal, LD—light-dark avoidance task, mPFC—medial prefrontal cortex, OBX-olfactory bulbectomy, p.o.—per os, STR—stressed group, s.c.—sub cutaneous, TST—tail suspension test, vHPC—ventral hippocampus.

pharmacological agents facilitating AEA activity is usually not accompanied by catalepsy, hypothermia and other effects that are associated with the administration of direct CB1 agonists (Kathuria et al., 2003; Jayamanne et al., 2006). One of the first available FAAH inhibitors was URB597, which is able to enhance AEA level in the brain without interacting in a significant manner with cannabinoid receptors (Tarzia et al., 2003; Mor et al., 2004; Fegley et al., 2005). The first studies about the impact of FAAH inhibitors on emotional behavior were published by Kathuria et al. (2003) and Gobbi et al. (2005) showing that increased AEA, but not 2-AG, brain levels produced by URB597 elicited anxiolytic- and antidepressant-like effects, respectively. The mood-elevating effects of URB597 were subsequently supported by several findings. Systemic or local administration of URB597 reduced the immobility in the FST (Hill et al., 2007; Adamczyk et al., 2008; Umathe et al., 2011) and TST (Naidu et al., 2007), without affecting motor activity, as assessed in the open field test; it was also able to counteract the effects of CMS paradigm in rodents (Bortolato et al., 2007; Rademacher & Hillard, 2007) and to reverse the depressive-like phenotype induced in adolescent female rats by Δ^9-THC exposure (Realini et al., 2011) (Table 3). These effects were almost always CB1-mediated, suggesting that the FAAH inhibition is followed by enhanced AEA signaling at CB1 receptors. Interestingly, the antidepressant-like effects of URB597 seem to be occurring in specific brain regions, since when CB1 receptors are specifically activated in the dentate gyrus of the HPC no effect could be observed (McLaughlin et al., 2007); by contrast, enhancement of intrinsic AEA levels in the medial PFC decreased passive coping behavior through regulation of 5-HTergic firing activity, further supporting that the potentiation of EC signaling facilitates monoaminergic neurotransmission (McLaughlin et al., 2012). The role of FAAH dysfunction in the pathogenesis of depressive disorders is also supported by the recent study of Vinod et al. (2012), showing that in Wistar Kyoto rats, a genetic model of depression, high levels of FAAH in brain regions such as the HPC and frontal cortex are associated with a depressive-like phenotype. More controversial outcomes have been reported on anxiolytic activity of URB597. Systemic or local (medial PFC or dlPAG) administration elicited anxiolytic responses in conditioned and unconditioned behavioral tests (Hill et al., 2009; Zanettini et al., 2011), a finding that was recently confirmed (Busquets-Garcia et al., 2011; Cippitelli et al., 2011). There are also conflicting reports of partial (Moise et al., 2008; Moreira et al., 2008; Haller et al., 2009; Micale et al., 2009a), or no anxiolytic responses (Naidu et al., 2007; Naderi et al., 2008) as well as of anxiogenic actions (Roohbakhsh et al., 2009; Seillier & Giuffrida, 2011), which in turn could be due to differences in the doses or in the aversiveness of testing conditions. Several side effects such as social withdrawal and cognitive deficits have been also found in rodents treated with URB597, which could be due to the fact that manipulating FAAH affect also other fatty acid ethanolamide (i.e. OEA and PEA), which in turn bind at non cannabinoid sites such as TRPV1 and PPAR-α (Seillier et al., 2010; Sokolic et al., 2011). The use of transgenic mice lacking FAAH enzyme can be advantageous to further evaluate the impact of increased endocannabinoid-CB1 signaling on emotional reactivity, despite potential compensatory mechanisms occurring during development (which represent a limitation of experiments with mutant mice in general). Since these mice are severely impaired in their capacity to degrade AEA, they exhibit more than 10-fold higher levels of this EC compared to wild types (WT) (Cravatt et al., 2001), while retaining normal brain CB1 density (Basavarajappa et al., 2006). In line with the pharmacological approach mentioned above, FAAH genetic inactivation induced in mice a less anxious- and depressive-like phenotype in some studies (Moreira et al., 2008; Bambico et al., 2010b) but not in others (Naidu et al., 2007). These discrepancies might be accounted for by the impact of different experimental conditions as well as by the differences in the strain in which FAAH mutant mice were backcrossed. In agreement with the psychotherapeutic efficacy in animal models of AEA degradation blockade, oleamide, a competitive inhibitor of FAAH, by elevating AEA concentration, decreased the immobility time in the FST, as index of antidepressant-like effects, through a CB1-mediated mechanism (Hill & Gorzalka, 2005; Akanmu et al., 2007), and also elicited anxiolytic-like effects in several behavioral tasks (Fedorova et al., 2001; Wei et al., 2007).

In the meantime additional selective FAAH inhibitors such as AACOCF3, PF-3845, AM3506 or JNJ5003 have been developed and are currently under intensive studies to assess their potential activity in emotional (Rutkowska et al., 2006; Kinsey et al., 2011; Gunduz-Cinar et al., 2012; Hill et al., 2012a) and non emotional behaviors (Feledziak et al., 2012) (Table 3). To confirm the preclinical data, the FAAH inhibitor PF-04457845 (Pfizer Inc) (Ahn et al., 2009), which failed to produce analgesia in a controlled clinical trial (Di Marzo, 2012b; Huggins et al., 2012), is currently being evaluated in a human clinical trial (Phase II) with the potential to treat fear response (ClinicalTrials.gov identifier: NTC01665573). As recently described, a particularly innovative approach could be the use of compounds with the capability to combine blockade of AEA hydrolysis with antagonism of TRPV1. These compounds should promote anxiolytic effects caused by activation of CB1 and at the same time prevent anxiogenic effects mediated by TRPV1 activation by elevated tissue levels of FAAH substrates with agonist activity at this channel (e.g. AEA, OEA and PEA). One such dual FAAH/TRPV1 blocker is N-arachidonoyl-serotonin (AA-5-HT) (Bisogno et al., 1998; Maione et al., 2007), which exerts antihyperalgesic effects by inactivating both proteins (Costa et al., 2010). Due to its property of dual indirect CB1 "enhancer" and TRPV1 blocker, AA-5-HT elicited anxiolytic- (Micale et al., 2009a,2009b; John & Currie, 2012) and antidepressant-like activity (Navarria et al., 2011), suggesting the potential therapeutic use of dual FAAH/TRPV1 inhibitors in affective disorders. These data further support the novel concept of dual acting agents as potentially useful tools in the treatment of stress-related disorders (Millan, 2009).

Another strategy to increase EC signaling at the receptor is to block the uptake of ECs into pre-and/or post postsynaptic terminals, thereby promoting the indirect activation of CB1 receptors. Unlike EC hydrolyzing enzymes, which have been fully identified and cloned, the functional properties of the putative membrane EC transporter such as the recent FAAH-like anadamide transporter (FLAT), have been only partially characterized (Hillard & Jarrahian, 2003; Yates & Barker, 2009; Fu et al., 2011) and its molecular identity remains still unknown. The prototypical EC transport inhibitor AM404 promoted fear-alleviating/anxiolytic effects in several behavioral tasks, which could be due in part to the direct activation of both CB1 and 5-HT_{1A} receptors as well as to FAAH inhibition (Bortolato et al., 2006; Patel & Hillard, 2006; Braida et al., 2007; Bitencourt et al., 2008; Abush & Akirav, 2010; Gomes et al., 2011a; Campolongo et al., 2012). Interestingly, local or systemic AM404 administration also improved the behavioral performance of rodents in the FST, through a CB1 mediated mechanism (Hill & Gorzalka, 2005; Adamczyk et al., 2008; Mannucci et al., 2011; Umathe et al., 2011) (Table 3). However, the exact mechanism of action of EC uptake inhibitors as well as of the molecular identity of the transporter itself still remain to be characterized, and therefore further biomolecular studies will have to be performed in this direction. Collectively, this evidence supports the clinical potential of EC level modulators as a new therapeutic tool for the treatment of the clinical conditions in which depressive and anxious symptoms are mixed together.

Since the expression of enzymes acting on 2-AG biosynthesis and degradation in brain regions controlling emotions are affected by environmental stressors such as maternal deprivation, cat odor, OBX (Sütt et al., 2008; Eisenstein et al., 2010; Suárez et al., 2010), 2-AG could act in the brain to modulate behavioral responses to stress-related conditions. However, little is known about the possible effects of 2-AG modulation in vivo in the emotional behavior. In this context, the recently developed highly selective MAGL inhibitors could be a promising tool to dissect 2-AG from AEA actions (Petrosino & Di Marzo, 2010). The prototypical MAGL inhibitor JZL184, by inducing an 8-fold increase in 2-AG, but not AEA, brain content elicited anxiolytic-like effects in highly aversive situations. The difference in the experimental approach (i.e.: high light vs low light intensity; systemic vs medial PFC injection) could be

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Table 3
Uptake and degradation inhibitors.

Drug	Treatment (days)	Effective dose (range tested)	Route[a]	Vehicle	Animals	Test[a]	Behavioral response	Positive control[a]	References
(a) FAAH inhibitors									
URB597	Acute	0.1/0.3 (0.03–0.3) mg/kg	i.p.	DMSO(1)/emulphor(1)/sal(18)	ICR mice	EPM	↓ anxiety	ND	Patel & Hillard, 2006
	Acute	(0.1–1–10) mg/kg	i.p.	Etoh(1)/alkamuls(1)/sal(18)	ICR/Bl6J mice	EPM	↔ anxiety	MDZ (1–2 mg/kg, i.p.)	Naidu et al., 2007
	Acute	0.1/0.3 (0.1–0.3) mg/kg	i.p.	Etoh(1)/emulphor(1)/sal(18)	Syrian hamsters	EPM	↓ anxiety	DZP (2 mg/kg, i.p.)	Moise et al., 2008
	Acute	1 mg/kg	i.p.	DMSO(1)/Etoh(1)/sal(18)	Bl6N mice	EPM/LD	↓ anxiety (EPM)/↔ (LD)	DZP (2 mg/kg, i.p.)	Moreira et al., 2008
	Acute	(0.03–0.3) mg/kg	i.p.	DMSO	NMRI mice	EPM	↔ anxiety	DZP (2–8 mg/kg, i.p.)	Naderi et al., 2008
	Acute	(0.1–0.3) mg/kg	i.p.	DMSO/0.4% methylcellulose/sal	SD rats	EPM	↔ anxiety	CDP (4–6 mg/kg, i.p.)	Haller et al., 2009
	Acute/chronic (7 d, 1/d)	1 (0.1–1) mg/kg	i.p.	10% DMSO/sal	Bl6J/Swiss mice	EPM	↓ anxiety	DZP (1 mg/kg, i.p.)	Micale et al., 2009a
	Acute	0.1/1 (0.01–1) μg/rat	vHPC	DMSO/Tw80/sal	Wistar rats	EPM	↑ anxiety	ND	Roohbakhsh et al., 2009
	Acute	0.1 (0.1–0.3) mg/kg	i.p.	5% PEG/5% Tw80/90% sal	Wistar rats	EPM	↓ anxiety in nicotine withdrawal	ND	Cippitelli et al., 2011
	Acute/chronic (6 d, 1/d)	1 mg/kg	i.p.	DMSO-15% DMSO/4.25% PEG/4.25% Tw80/sal	Swiss/Bl6J mice	EPM/FC	↓ anxiety/↓ freezing	ND	Busquets-Garcia et al., 2011
	Acute	0.3 (0.1–1) mg/kg	i.p.	5% PEG/5% Tw80/90% sal	Wistar rats	EPM	↑ anxiety in PCP rats	DZP (1 mg/kg, i.p.)	Seillier & Giuffrida, 2011
	Acute/chronic (4 d, 1/d)	0.1/0.3 (0.03–0.3) mg/kg	i.p.	5% PEG/5% Tw80/90% sal	Bl6 mice/Wistar rats	TST/FST	↓ immobility	DMI (20 mg/kg, i.p.), PAR (10 mg/kg, i.p.)	Gobbi et al., 2005
	Acute	0.3 mg/kg	i.p.	DMSO(1)/emulphor(1)/sal(8)	ICR mice	SPT	↑ sucrose consumption in STR mice	ND	Rademacher & Hillard, 2007
	Acute	0.1/(0.1–0.3) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(8)	LE rats	FST	↓ immobility	ND	Hill et al., 2007
	Repeated (1 d, 3/d)	(0.5–1) μg/side	dHPC	DMSO	SD rats	FST	↔ immobility	ND	McLaughlin et al., 2007
	Acute	0.1 mg/kg	i.p.	Tw80(1)/PEG(1)/sal(18)	Bl6J mice	TST	↓ immobility	DMI (15 mg/kg, i.p.)	Naidu et al., 2007
	Chronic (35 d, 1/d)	0.4 (0.03–0.3) mg/kg	i.p.	5% PEG/5% Tw80/90% sal	Wistar rats	CMS	↑ sucrose consumption in STR rats	IMI (20 mg/kg, i.p.)	Bortolato et al., 2007
	Acute	0.1/0.3 (0.03–0.3) mg/kg	i.p.	Etoh/Tw80/sal	Wistar rats	FST	↓ immobility	ND	Adamczyk et al., 2008
	Acute	0.05/0.1/1/5/10 (0.01–10) μg/mouse	i.c.v.	DMSO(2)/Tw80(1)/sal(7)	Swiss mice	FST/MBB	↓ immobility/↓ anxiety	FLU (5–20 mg/kg, i.p.)	Umathe et al., 2011
	Chronic (30 d, 1/d)	0.3 mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(8)	SD rats	FST/SPT	↓ immobility/↑ sucrose consumption in Δ^9-THC rats	ND	Realini et al., 2011
	Acute	0.01 μg/side	vmPFC	DMSO/sal	SD rats	FST	↓ immobility	ND	McLaughlin et al., 2012
Oleamide	Acute	5 mg/kg	i.p.	5% DMSO/20% alkamuls/75% w	SD rats	EPM	↓ anxiety	ND	Fedorova et al., 2001
	Acute	10/20 (5–20) mg/kg	i.p.	Corn oil	Swiss mice	EPM/LD	↓ anxiety	DZP (2.5 mg/kg, i.p.)	Wei et al., 2007
	Repeated (1 d, 3/d)	2.5/5 (1–5) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	LE rats	FST	↓ immobility	DMI (10 mg/kg, i.p.)	Hill & Gorzalka, 2005
	Repeated (1 d, 3/d)	10 (5–10) mg/kg	i.p.	10% Etoh/Tw80/sal	Albino mice	FST	↓ immobility	ND	Akanmu et al., 2007
AACOCF3	Acute	4 (1–4) mg/kg	i.p.	Crem(1)/sal(18)	BALB/c mice	LD	↓ anxiety	ND	Rutkowska et al., 2006

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PF-3845	Acute	10 (1–10) mg/kg	i.p.	Etoh(1)/alkamuls(1)/sal(18)	Bl6J	MBB	↓ anxiety	DZP (1 mg/kg, i.p.)	Kinsey et al., 2011
AM3506	Acute	0.25/0.5/1 (0.25–1) mg/kg-0.1 μg/side	i.p./BLA	DMSO(1)/sal(18)	S1 mice	FC	↓ freezing	ND	Gunduz-Cinar et al., 2012
	Acute	(1) mg/kg	i.p.	DMSO(1)//sal(18)	S1 mice	FST	↔ immobility	ND	Gunduz-Cinar et al., 2012
JNJ5003	Chronic (21 d, 1/d)	50 mg/kg/day	p.o. (chow)	ND	Bl6 mice	CMS	↓ anxiety in the EPM	ND	Hill et al., 2012a
(b) FAAH inhibitor/TRPV1 blockade									
AA-5-HT	Acute/chronic (7 d, 1/d)	0.1/0.5/1/2.5 (0.1–5) mg/kg	i.p.	10% DMSO/sal	Bl6J/Swiss mice	EPM	↓ anxiety	DZP (1 mg/kg, i.p.)	Micale et al., 2009a
	Acute	0.1/0.5/1/2.5 (0.1–5) mg/kg	i.p.	10% DMSO/sal	D3R-KO/WT mice	EPM	↓ anxiety (WT)	DZP (1 mg/kg, i.p.)	Micale et al., 2009b
	Acute	0.25/0.5 (0.125–0.5) nmol/side	BLA	25% DMSO/sw	SD rats	EPM	↓ anxiety	ND	John & Currie, 2012
	Repeated (1 d, 3/d)	5 (2.5–5) mg/kg	i.p.	10% DMSO/sal	Wistar rats	FST	↓ immobility in STR rats	CLM (50 mg/kg, i.p.)	Navarria et al., 2011
(c) AEA uptake inhibitors									
AM404	Acute	5 (0.5–5) mg/kg	i.p.	PEG(5)/Tw80(5)/sal(90)	Wistar rats	EPM	↓ anxiety	DZP (2.5 mg/kg, i.p.)	Bortolato et al., 2006
	Acute	1/3 (0.3–10) mg/kg	i.p.	Etoh(1)/emulphor(1)/sal(18)	Swiss mice	EPM	↓ anxiety	ND	Patel & Hillard, 2006
	Acute	0.75/1.25 (0.015–1.25) mg/kg	i.p.	10% DMSO/sal	SD rats	EPM	↓ anxiety	ND	Braida et al., 2007
	Acute	1/2 (0.25–2) mg/kg	i.p.	18% DMSO/1% Etho/1% emulphor/80% sal	NMRI mice	EPM	↓ anxiety	DZP (2–8 mg/kg, i.p.)	Naderi et al., 2008
	Acute	1 (0.2–2) μg/rat	i.c.v.	10% DMSO/0.1 M PBS/sal	Wistar rats	FC	↓ freezing	DZP (2.85 μg/rat, i.c.v.)	Bitencourt et al., 2008
	Acute	1/3 (0.3–3) mg/kg	i.p.	Tocrisolve TM 100/sal	Bl6J mice	MBB	↓ anxiety	ND	Gomes et al., 2011a
	Repeated (1 d, 3/d)	5 (1–5) mg/kg	i.p.	DMSO(1)/Tw80(1)/sal(18)	LE rats	FST	↓ immobility	DMI (10 mg/kg, i.p.)	Hill & Gorzalka, 2005
	Acute	0.3/1/3 (0.1–3) mg/kg	i.p.	Tocrisolve TM 100/dw	Wistar rats	FST	↓ immobility	IMI (30 mg/kg, i.p.)	Adamczyk et al., 2008
	Acute	0.5/1/2 (0.5–2) mg/kg	s.c.	sal	Swiss mice	FST	↓ immobility	ND	Mannucci et al., 2011
	Acute	0.1/1/5/10 (0.05–10) μg/mouse	i.c.v.	DMSO(2)/Tw80(1)/sal(7)	Swiss mice	FST	↓ immobility	FLU (5–20 mg/kg, i.p.)	Umathe et al., 2011
VDM11	Acute	100 pmol/side	dHPC	0.1% DMSO/sal	Wistar rats	EPM	↔ anxiety	ND	Clarke et al., 2008
(d) MAGL inhibitors									
JZL184	Acute/chronic (6 d, 1/d)	8 mg/kg	i.p.	DMSO-15% DMSO/4.25% PEG/4.25% Tw80/sal	Swiss/Bl6J mice	EPM/FC	↓ anxiety/↔ freezing	ND	Busquets-Garcia et al., 2011
	Acute	16 (4–40) mg/kg,	i.p.	Etoh(1)/alkamuls(1)/sal(18)	Bl6J mice	MBB	↓ anxiety	DZP (1 mg/kg, i.p.)	Kinsey et al., 2011
	Acute	8 (1–8) mg/kg	i.p.	20% DMSO/80% Emulphor(1)/Etoh(1)/sal(8)	SD rats	EPM	↓ anxiety (highly aversive environment)	DZP (1 mg/kg, i.p.)	Sciolino et al., 2011
	Acute	(4–8–16) mg/kg	i.p.	DMSO/0.4% methylcellulose/sal	CD1/Bl6J mice	EPM	↔	ND	Aliczki et al., 2012
URB602	Acute	10 mg/kg	i.p.	2% DMSO/2% crem/sal	SD rats	FST	↓ immobility in DFP intoxicated rats (combined with URB597 3 mg/kg)	Atropine (16 mg/kg, i.p.)	Wright et al., 2010

[a] BLA—basolateral amygdala, CDP—chlordiazepoxide, CLM—clomipramine, CMS—chronic mild stress, DFP—diisopropylfluorophosphate, dHPC—dorsal hippocampus, DMI—desipramine, DZP—diazepam, EPM—elevated plusmaze, FC—fear conditioning, FLU—fluoxetine, FST—forced swim test, i.c.v.—intracerebroventricular, IMI—imipramine, i.p.—intraperitoneal, LD—light-dark avoidance task, MDZ—midazolam, MBB—marble burying behaviour, PAR-paroxetine, PCP—phencyclidine, p.o.—per os, s.c.—sub cutaneous, SPT—sucrose preference test, STR-stressed group, TST—tail suspension test, vHPC—ventral hippocampus, vmPFC—ventramedial prefrontal cortex.

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Table 4
Non-psychotropic cannabinoids.

Drug	Treatment	Effective dose (range tested)	Route[a]	Vehicle	Animals	Test[a]	Behavioral response	Positive control[a]	References
Cannabidiol	Acute	2.5/5/10 (2.5–20) mg/kg	i.p.	1% Tw80/10% PEG/sal	Wistar rats	EPM	↓ anxiety	DZP (2 mg/kg, i.p.)	Guimarães et al., 1990
	Acute	10 mg/kg	i.p.	2% Tw80/sal	Wistar rats	FC	↓ freezing	DZP (2.5 mg/kg, i.p.)	Resstel et al., 2006
	Acute	2 (0.2–2) μg/rat	i.c.v.	10% DMSO/0.1 M PBS/sal	Wistar rats	FC	↓ freezing	DZP (2.85 μg/i.c.v.)	Bitencourt et al., 2008
	Acute	30 (15–60) nmol/rat	dlPAG	Grape seed oil	Wistar rats	EPM	↓ anxiety	ND	Campos & Guimarães, 2008
	Acute/chronic (7 d, 1/d)	15/30/60 (5–60) mg/kg	i.p.	Grape seed oil	Bl6J mice	MBB	↓ anxiety	DZP (2.5 mg/kg, i.p.); PAR (10 mg/kg, i.p.)	Casarotto et al., 2010
	Acute	30 (15–60) nmol/side	plPFC/ilPFC	Grape seed oil	Wistar rats	FC	↓ freezing (plPFC)/↑ freezing (ilPFC)	ND	Lemos et al., 2010
	Chronic (17 d, 1/d)	1 (1–50) mg/kg	i.p.	Etoh(1)/Tw80(1)/sal(18)	Bl6JArc mice	LD/EPM	↓ anxiety (LD)/↔ (EPM)	ND	Long et al., 2010
	Acute	60 (15–60) nmol/side	BNST	Grape seed oil	Wistar rats	EPM	↓ anxiety	ND	Gomes et al., 2011b
	Acute	30 nmol/rat	i.c.	Grape seed oil	Wistar rats	EPM	↓ anxiety in STR rats	ND	Granjeiro et al., 2011
	Acute	120 mg/kg	i.p./p.o.	Etoh(1)/crem(1)/sal(18)	Swiss mice	MBB	↓ anxiety	ND	Deiana et al., 2012
	Acute	30/60 (15–60) nmol/side	BNST	Grape seed oil	Wistar rats	FC	↓ freezing	ND	Gomes et al., 2012
	Acute	1 (0.5–1) μg/rat	CeA	2% DMSO	Wistar rats	EPM	↓ anxiety	ND	Hsiao et al., 2012
	Acute/chronic (15 d, 1/d)	100 (1–100) mg/kg	i.p.	Etoh(1)/Tw80(1)/sal(18)	Nrg1 TM HET/WT mice	LD	↓ anxiety in Nrg1 TM HET mice	ND	Long et al., 2012
	Acute/chronic (7 d, 14 d, 1/d)	2.5 mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	SD rats	LD	↔ anxiety	ND	O'Brien et al., 2013
	Acute	3/10/30 (3–30) mg/kg	i.p.	5% Tw80/sal	Wistar rats	FC	↓ freezing	MDZ (1.5 mg/kg, i.p.)	Stern et al., 2012
	Acute	200 (20–200) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	Swiss-DBA/2 mice	FST/TST	↓ immobility (FST)/↔ (TST)	FLU (40 mg/kg, i.p.); DMI (20–40 mg/kg, i.p.)	El-Alfy et al., 2010
	Acute	30 (3–100) mg/kg	i.p.	2% Tw80/sal	Swiss mice	FST	↓ immobility	IMI (30 mg/kg, i.p.)	Zanelati et al., 2010
Cannabichromene	Acute	20/40/80 (20–80) mg/kg	i.p.	Etoh(1)/crem(1)/sal(18)	Swiss-DBA/2 mice	FST/TST	↓ immobility	FLU (40 mg/kg, i.p.); DMI (20–40 mg/kg, i.p.)	El-Alfy et al., 2010

[a] BNST—bed nucleus of the stria terminalis, CeA—central amygdala, dlPAG—dorsolateral periaqueductal grey, DMI—desipramine, DZP—diazepam, EPM—elevated plusmaze, FC—fear conditioning, FLU—fluoxetine, FST—forced swim test, IMI—imipramine, ilPFC—infralimbic prefrontal cortex, i.c.—intracisternal; i.c.v.—intracerebroventricular, i.p.—intraperitoneal, LD—light-dark avoidance task, MBB—marble burying behavior, MDZ—midazolam, PAR—paroxetine, plPFC—prelimbic prefrontal cortex, p.o.—per os, STR-stressed group, TST—tail suspension test.

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the reason why in the study of Rubino et al. (2008b) 2-AG treatment failed to produce anxiolytic response in rodents. However, we still do not know exactly which cannabinoid receptor could be involved in this effect, since it may to be mediated by either CB1 or CB2 receptors, or both (Busquets-Garcia et al., 2011; Kinsey et al., 2011; Sciolino et al., 2011; Aliczki et al., 2012). To the best of our knowledge, there is only one report on the antidepressant-like action of this class of compounds. More specifically, Wright et al. (2010) showed that the MAGL inhibitor URB602 (which, however is weak as well as unselective), in combination with the FAAH inhibitor URB597, but not per se, reversed the enhanced immobility time in an animal model of organophosphorus intoxication. Although more studies are clearly needed, these initial results suggest that the pharmacological inhibitors of MAGL could possess antidepressant properties. However, contrary to FAAH blockade, a potential drawback in the use of MAGL inhibitors could be the development of tetrad effects which are typical of CB1 agonists (Long et al., 2009) as well as of tolerance with chronic use (Schlosburg et al., 2010). In conclusion, while ECs are rapidly metabolized in vivo, limiting the potential efficacy of their exogenous administration, the data described above support more FAAH than MAGL as potential therapeutic targets for the identification of new pharmacotherapies for psychiatric disorders.

4.4. Non-psychotropic cannabinoids and mood disorders

In addition to the pharmacological modulation of the ECS, a different approach to minimize the psychotropic side effects of *Cannabis* is the use of phytocannabinoids with very weak or no psychotropic effects. These include cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), D9-tetrahydrocannabivarin (D9-THCV) and cannabidivarin (CBDV), some of which show potential as therapeutic agents in preclinical models of CNS disorders (Hill et al., 2012b). Here, as described in Table 4, we specifically restrict our discussion to recent developments in the preclinical pharmacology of non-psychotropic phytocannabinoids for possible therapeutic use in the treatment of stress-related disorders. One of the most promising candidates of this class of seemingly safe compounds is CBD, which exerts several positive pharmacological effects in preclinical and clinical studies to the point of making it a highly attractive therapeutic entity in several diseases. We still do not know the exact mechanism(s) of action underlying the mood-elevating effect of CBD, but this compound could not only act through the ECS, but also directly or indirectly activate the metabotropic receptors for 5-HT or adenosine, or target nuclear receptors of the PPAR family as well as modulating ion channels including TRPV1 (Izzo et al., 2009). In an experimental model of unconditioned anxiety, CBD showed bidirectional effects with low to moderate doses (from 2.5 to 10 mg/kg) being anxiolytic (Guimarães et al., 1990), with similar effect sizes as shown by diazepam, the prototypic anxiolityc compound. This effect was further confirmed by several subsequent studies in which the systemic (Moreira et al., 2006; Resstel et al., 2006; Malone et al., 2009; Casarotto et al., 2010; Long et al., 2010; Deiana et al., 2012; Stern et al., 2012) or local (i.c.v. or cisterna magna) (Bitencourt et al., 2008; Granjeiro et al., 2011) administration of CBD decreased anxiety-like behavior in rodents. Interestingly, this effect seems to target a specific brain region: injection of CBD into the dlPAG, the bed nucleus of the stria terminalis (BNST), the prelimbic PFC (plPFC) or the central nucleus of the amygdala (CeA) exerted anxiolytic effects (Campos & Guimarães, 2008; Lemos et al., 2010; Soares et al., 2010; Gomes et al., 2011b, 2012; Hsiao et al., 2012); by contrast, injection of CBD into the infralimbic PFC (ilPFC) increased anxiety (Lemos et al., 2010). Recent clinical studies have confirmed the anxiolytic properties of CBD in humans, through an activity in limbic and paralimbic brain areas (Bergamaschi et al., 2011a; Crippa et al., 2011). In the FST but not in the TST, which represent standard preclinical tests to assess the effects of potential antidepressants, CBD decreased the immobility time through a 5-HT_{1A}-mediated mechanism (El-Alfy et al., 2010; Zanelati et al., 2010). Furthermore, several studies suggest an antipsychotic effect of CBD in humans (Ashton & Moore, 2011). For example, in a recent double-blind, randomized clinical trial of CBD vs amisulpride, a potent antipsychotic, by Leweke et al. (2012), the authors reported that treatment with CBD (800 mg/day) led to significant clinical improvement and displayed a markedly superior side-effect profile. Moreover, in agreement with its previously reported inhibition of FAAH (Bisogno et al., 2001), CBD treatment was accompanied by a significant increase in serum AEA levels, which was significantly associated with clinical improvement. The authors suggest that inhibition of AEA deactivation may contribute to the antipsychotic effects of CBD. However, in a small human trial, CBD administration failed to improve the symptoms in patients suffering of bipolar disorder (Zuardi et al., 2010). Thus, further studies are needed to assess both the effects of CBD in depressive disorders and its safety profile, although this latter is already well supported (Bergamaschi et al., 2011b; Wade, 2012).

Of the other non psychotropic phytocannabinoids, cannabichromene but not Δ^8-THC, cannabinol or cannabigerol, improved the behavioral performance of mice in the FST (El-Alfy et al., 2010). Further studies to assess the potential mood elevating effects of others phytocannabinoids are clearly necessary.

5. Future prospective and conclusive remarks

In conclusion, whilst the direct modulation of CB1 receptors for the treatment of mood disorders is hampered by unwanted psychotropic effects, and the possibly safer direct modulation of CB2 receptors still lacks sufficient experimental evidence to justify its use (Fig.1A), the indirect activation of cannabinoid receptors with agents that inhibit ECs deactivation has produced very promising results in animal models of anxiety- and depression-like signs (Fig. 1B). However, even this approach, which resembles to somehow that used with SSRI drugs, has its problems, mostly due to the fact that ECs-deactivating proteins also recognize as substrates other non-endocannabinoid mediators which then activate different receptors (a property shared to some extent also by endocannabinoids like AEA and NADA). Thus, inhibition of enzymes like FAAH or of the putative EC transporter might lead to activation of these alternative receptors. This complication, as well as the possible compensatory action of co-occurring deactivation routes and enzymes for ECs (see Piscitelli & Di Marzo, 2012 for a recent review), may render this approach not sufficiently efficacious or safe. In view of these potential problems, medicinal chemists and pharmacologists are now exploring also the possible use of multi-target drugs (such as, for example, dual FAAH-TRPV1 blockers) and of non-psychotropic cannabinoids such as CBD. Only time will tell if these pre-clinical studies will bring us some new much needed pharmacotherapies for anxiety, depression and other affective disorders.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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