The Critical Role of the Endocannabinoid System in Emotional Homeostasis: Avoiding Excess and Deficiencies

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Abstract: The endocannabinoid system is critical in the regulation of emotion and stress responsiveness. Despite the promising therapeutic value of its pharmacological modulation, deficient and excessive endocannabinoid signalling should be avoided. This mini-review will provide an up-to-date revision on this topic, emphasizing the relevance of a normative endocannabinoid system for emotional homeostasis.

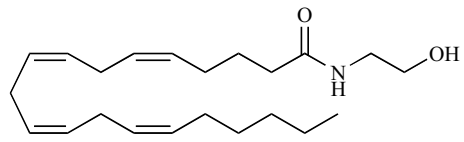
Key Words: Neuropharmacology, endocannabinoids, animal behavior, mental disorders.

1. INTRODUCTION

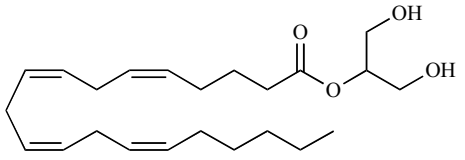
In the last decades, the endocannabinoid system (ECS) has arisen as a major neuromodulatory system critically involved in the regulation of emotional states. Complex effects of cannabis and cannabinoid agonists on anxiety-related behaviour have been described in both human and animal studies [1]. The brain distribution of cannabinoid type-1 receptors (CB1R), with high densities in cortical and limbic structures, is consistent with its involvement in the regulation of emotional reactivity [2]. In addition, an altered emotional phenotype is observed in mice lacking CB1R (CB1 knockout mice), namely, increased anxiety levels and depressive-like symptoms accompanied by an aberrant hypothalamus-pituitary-adrenal (HPA) axis activity. This particular role of the ECS in emotional control has other obvious implications. Deregulation or malfunctioning of the ECS might contribute to the aetiology of several human diseases, including neuropsychiatric disorders such as anxiety disorders, depression and schizophrenia [3-6]. Alternatively, the pharmacological modulation of the ECS has arisen as a promising therapeutic tool. In particular, augmentation of the ECS signalling through inhibition of endocannabinoid deactivation may promote stress-coping behaviour, thus providing a potential pharmacological strategy notably relevant for its potential analgesic, anxiolytic and anti-depressant properties [7]. However, the ubiquitous and multifunctional nature of the ECS discourages its manipulation under diverse circumstances. In summary, appropriateness and accuracy in endocannabinoid function seem essential for an adequate response to stressful and/or aversive situations. This mini-review will focus on the critical relevance of keeping the endocannabinoid tone within physiological limits in order to assure a psycho-physiological equilibrium.

2. THE ENDOCANNABINOID SYSTEM (ECS)

The ECS is composed by 1) the endogenous ligands, polyunsaturated fatty acid derivatives named as endocannabinoids, 2) the enzymatic machinery in charge of their synthesis and inactivation and 3) specific membrane receptors. Endocannabinoids, due to their lipophilic nature, are synthesized and released 'on demand' by the cleavage of membrane phospholipid precursors in response to diverse physiological and pathological stimuli. Among endocannabinoids, research has mainly focused on anandamide, an ethanolamide of arachidonic acid (AEA) and 2-arachidonoylglycerol (2-AG) (Fig. **1**) [8-10]. Deactivation processes strictly regulate action of endocannabinoids: carrier-mediated uptake into neurons and glia, followed by intracellular hydrolysis. Endocannabinoids can passively diffuse through lipid membranes, but a high affinity transporter, apparently different for AEA and 2-AG although not yet identified, seems to accelerate this process [11,12]. On the one hand, anandamide inactivation is completed by the activity of the intracellular fatty acid amide hydrolase (FAAH) [13], which recognizes as substrates both AEA and 2-AG but seems to be more often involved in AEA inactivation. On the other hand, the primary route for 2-AG inactivation is afforded by the enzime monoacylglycerol lipase (MGL) [14,15], although novel 2-AG-hydrolysing lipases have been very recently identified [16]. The biochemical scenario of endocannabinoids' synthesis and/or metabolism is far more complex, and



Anandamide (AEA)



2-arachidonoylglycerol (2-AG)

Fig. (1). Chemical structure of the main endocannabinoid ligands, anandamide (AEA) and 2-arachidonoylglycerol (2AG).

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the molecular characterization of all these proteins may critically contribute to advances in the field. For further information please refer to several recent review papers which would provide a more appropriately detailed analysis of these processes [17,18].

Endocannabinoids have been shown to modulate neurotransmission, mainly acting as retrograde transmitters [19], and have been reported to be at the basis of a plethora of physiological processes, including appetite, pain modulation, motivation, cognition, and control of emotional and stress-related responses [1,20-23]. Endocannabinoids mainly act through activation of specific metabotropic receptors coupled to $G\alpha_{i/o}$ proteins (cannabinoid receptor type 1 and 2, CB1R and CBR2, respectively). CB1Rs are widely expressed in the central nervous system and they are found at highest concentrations in the hippocampus, neocortex, basal ganglia and cerebellum; while a moderate presence is observed in the basolateral amygdala, hypothalamus, and midbrain [2]. CB2Rs, were initially thought to be localized exclusively in peripheral immune tissues, in contrast, they are also present in neuronal and glial cells within the central nervous system [24,25]. Not to forget, the existence of alternative targets of endocannabinoid ligands that could also participate in the above mentioned ECS functions. The transient receptor potential vanilloid type 1 (TRPV1) ion channel has been reported to mediate some AEA effects [26]. The peroxisome proliferators-activated nuclear receptor [27] and the G protein-coupled GPR55 receptor [28] have also been indicated as possible targets of endocannabinoid compounds.

3. EVIDENCES FOR A ROLE OF THE ECS IN EMOTIONAL CONTROL

There is substantial evidence from both human and animal studies for a role of the ECS in the control of emotional states. Firstly, the ECS is widely distributed in brain areas associated with emotional regulation and stress responsiveness. Regions with significant CB1R density include the hippocampus, prefrontal cortex, amygdale, hypothalamus and midbrain monoaminergic nuclei such as the locus coeruleus and dorsal raphe [2]. The ECS may modulate neurotransmitter release through activation of CB1Rs, and therefore regulate neuronal activation in stress-sensitive anatomical circuits.

Increasing evidence from human and animal behaviour studies supports the involvement of the ECS in emotional regulation. In humans, cannabis consumption is mostly associated with the experience of euphoriant feelings, widely known as 'high', that are usually accompanied by anxiolytic and pro-social effects. However, opposite effects such as dysphoric reactions, anxiety, panic, and psychosis have also been reported following recreational cannabis [29-34]. A similarly complex scenario has been reported in rodents, where a bidirectional profile of action regarding anxiety-like responses is observed upon administration of cannabis derivatives. In general, low doses seem to produce anxiolytic-like effects, while dosage increments seem to turn these effects into anxiogenic-related responses. Low doses of cannabinoid agonists have been reported to induce anxiolytic-like responses in classical behavioural paradigms, whereas mid to high doses seem to promote anxiogenic-like responses (See for review [1,21,23]). However, these biphasic effects are also contingent on other factors, including animal specie and strain, sex, age, basal emotional state, testing environment and previous experience with the drug (See for review [1,22]). Despite the great efforts devoted to understanding the biphasic profile of cannabinoid-induced effects, not exclusive of emotional-related responses, a consensus on the underlying mechanisms has not been reached (See for review [1,21]).

Genetic and pharmacological blockade of CB1Rs further support the role of the ECS in emotional regulation. Mutant mice lacking CB1Rs displayed higher anxiety levels in diverse behavioural paradigms, (i.e. the light-dark box, the elevated plus-maze test and the social interaction test), and increased aggressiveness as measured in the resident-intruder test, that were accompanied by profound alterations in adrenocortical activity [35-37]. Discrepant findings were also reported [38,39], but were probably a consequence of differences on the basal emotional state of animals. Baseline trait levels of emotionality are critically influenced by both the genetic strain and environmental testing conditions [40, 41]. In this regard, mutant CB1R mice exclusively exhibited an anxious phenotype under aversive conditions, i.e. high illumination and first exposure in the elevated plus-maze and the social interaction test [35,36,38,39]. Furthermore, the pharmacological blockade of CB1Rs by the administration of antagonists, e.g. rimonabant (SR141716A), produced anxiogenic-like effects in rats [42,43], although discrepancies also arise. In particular, rimonabant displayed anxiolytic-like properties in mice exposed to the plus-maze [35]. Neither dose-dependent nor biphasic anxiety-related effects of rimonabant have been reported to date [i.e. studies usually consider a single drug dose (1 or 3 mg/kg)]. In this occasion, basal anxiety levels, due to environmental and/or genetic differences (specie/strain), may principally account for the discrepant results described in literature. Actually, no behavioural effect of rimonabant was observed in naïve mice exposed for the first time to the elevated plus-maze, whilst an anxiolytic-like response was evidenced whenever mice had been previously exposed to the maze [44]. Taken together, the above-discussed findings support a role for the ECS in the control of emotional states, and may suggest the existence of an anxiolytic endogenous cannabinoid tone.

4. ROLE OF THE ECS IN STRESS RESPONSIVENESS

Upon exposure to stressful stimuli, cascades of physiological responses occur in order to enable adaptation to the particular situation. Among those, endocannabinoid-mediated plasticity seems to play a crucial role [3]. Transient changes in endocannabinoid content have been observed following experimental conditions that resemble acute aversive and/or stressful stimuli. An increase in AEA levels was found in response to pain, particularly in periaqueductal grey (PAG) microdialysates following formalin injection in rat paw [45]. In rats exposed to food deprivation both AEA and 2-AG content were found to increase in the nucleus Accumbens (NAcc), while only 2-AG levels augmented within the hypothalamus [46]. Similar results were found in fear-conditioned animals during exposure to the conditioned stimulus, i.e. acoustic tone that had been previously associated to an electrical foot-shock. In this occasion, a transient increase of

AEA levels in the basolateral amygdala was described [39]. Available evidence suggests that an activation of the ECS within limbic areas occurs in response to stress, however, opposite results were obtained when prolonged immobilization was used as stressor. A single episode of immobilization restraint (30 min.) causes a reduction in hypothalamic 2-AG content, and in AEA levels within amygdala and hippocampus. In addition, this single episode of restraint also produced an increase in plasma corticosterone levels. In contrast, the repeated exposure to this particular stressor (5 days of 30 min. restraint) increased 2-AG levels and reduced plasma corticosterone concentration [47]. Given the inhibitory role of CB1Rs on corticosterone biosynthesis and release [20], some authors have hypothesised that the stress-induced reduction of endocannabinoid levels may serve to disinhibit corticosterone release, that may therefore contribute to improve stress-coping behaviours. On contrast, the elevation of endocannabinoid levels upon re-exposure to a homotypic stressor may possibly mediate the observed inhibition of corticosterone release [3,47]. Taken together, endocannabinoid changes may underlie some of the emotional responses to stress. The ECS seems to participate in stress-coping situations, thus acting as a *stress-recovery* system, although an additional role in adaptation to similar stressful situations has been proposed. However, whether the ECS mediates recovery and/or adaptation to stressful situations is still unclear.

It is worth mentioning that whenever the stressful situation spans over time, becoming maybe chronic, the outcomes shall be deleterious. In fact, situations of chronic stress have been related to the pathogenesis of depression. In rodents, chronic exposure to stressful situations [i.e. chronic unpredictable stress (CUS) and chronic mild stress (CMS)], are procedures employed to investigate some of the symptoms present in the aetiology of depression such as anhedonia, learned helplessness and cognitive impairments [48]. Increasing evidence from both humans and animal models indicates that the ECS is changed during depression (for review see [5]). In summary, although the underlying biochemical mechanisms have not been fully established, plastic changes of the ECS seem to occur in brain areas involved in emotionality and may participate in recovery from stress, with obvious implications for the treatment of anxiety and mood-related disorders.

5. MODULATING THE ECS: AVOIDING EXCESS AND DEFICIENCY

Natural products and synthetic agents targeting the ECS has long been used in the management of diverse pathological conditions. Medicinal cannabis, cannabis extracts, and other cannabinoids are currently in use or under clinical trial investigation for the control of nausea, emesis and wasting in patients undergoing chemotherapy, the control of neuropathic and arthritic pain, and the control of certain symptoms of multiple sclerosis. Modulation of CB1R signalling, by direct or indirect agonists, as well as antagonists, can produce effects in stress response and mood-related behaviour. At present, pharmacological modulation of the ECS basically consists of its blockade through application of CB1R antagonists, or of magnifying tonic actions of endocannabinoids by inhibiting their deactivation processes.

5.1. Blockade ECS Signalling

Pharmacological blockade of CB1R has been proposed of therapeutical value in the treatment of obesity and associated metabolic disorders, and several selective CB1R antagonists with varied chemical structures have been tested in preclinical or clinical trials [49,50]. Since hyperactivity of the ECS was described to underlie the aetiology of obesity, application of high-affinity, selective CB1R ligands to attenuate endocannabinoid signalling appeared as a promising approach in obesity pharmacotherapy [51]. Brain ECS appears to control both food intake and energy balance [50, 52], and CB1R blockade has been shown to induce anorectic actions and improve metabolic parameters in several animal models [53-55]. According to evidence, in July 2006, European regulatory authorities approved the use of rimonabant, commercialized as Acomplia® by Sanofi-Aventis, in the management of obese patients. Despite the extensive and promising clinical trial data, doubts on drug safety appeared, and it was last October (2008) that Rimonabant was withdrawn from the European market due to the adverse psychiatric side effects observed in clinical trials, mostly anxiety, depression and suicidal ideations [56,57].

Pharmacological blockade of CB1R has also been proposed as a promising therapeutic strategy in the management of drug dependence, and more particularly in the management of nicotine dependence [49,58]. Functional interactions between nicotine and the ECS have been extensively studied [59]. Changes in both brain endocannabinoid content and CB1R expression have been described following nicotine administration [60,61]. Furthermore, findings from animal studies indicated that CB1R blockade might be effective not only as an aid for smoking cessation but also in the maintenance of abstinence. Rimonabant administration did not only reduce nicotine self-administration, but also prevented relapse [62,63]. Accordingly, Rimonabant confirmed its efficacy in the management of smoking cessation and relapse in several clinical trials. However, Rimonabant's benefits seem not to exceed those of nicotine-replacement therapy, and, in any case, the pharmacological blockade of CB1Rs may be limited by its psychiatric side effects (see above) [64,65].

Emerging preclinical data suggest that therapeutic attenuation of CB1R transmission shall benefit from the development of not centrally acting CB1R antagonists that may represent a highly promising tool in obesity management [54], and from CB1R neutral antagonists that may represent breakthrough medications of superior efficacy than antagonists/inverse agonists such as Rimonabant [56,57].

5.2. Enhancing ECS Signalling

In the recent years, novel pharmacological tools that promote endocannabinoid signalling have been developed. These compounds, also known as endocannabinoid enhancers or indirect cannabinoid agonists, specifically target the enzymatic machinery in charge of endocannabinoid degradation, thus enduring endocannabinoid activity while preserving its spatiotemporal specificity. Nowadays, a great variety of these compounds are already available for preclinical studies. AEA elimination is prevented by transport inhibitors such as AM404 [11], UCM707 [66], OMDM-1 and OMDM-2 [67], and VDM11 [68]; as well as by inhibi-

tors of AEA hydrolysis such as URB597 [7,68,69] and OL-135 [70]. Even less extensively investigated, a few inhibitors of 2-AG hydrolysis have been reported such as URB602, a non-compe-titive and partially reversible inhibitor of MGL [71,72]. This pharmacological approach seems to provide benefits over administration of direct non-selective cannabinoid agonists; for instance, it might provide fewer side effects.

Anxiolytic and antidepressant properties of cannabinoid compounds have been evaluated in animal models by comparison with a reference compound, that is, drugs used in the clinic. An increasing body of evidence consistently indicates that enhancement of AEA signalling promotes active stress-coping behaviours and exerts notable anxiolytic and antidepressant effects in rodents (Table **1**). URB597 administration produces anxiolytic-like responses in adult rats exposed to the light-dark test and to the elevated zero-maze [69,73], and in rat pups as evidenced by the reduction in the frequency of ultrasonic vocalizations induced by isolation [69]. Similarly, AM404 administration induced anxiolytic-like effects in the elevated plus-maze in rats [74], although no effects were observed when testing mice in the light-dark test box [75]. URB597 has also shown antidepressant properties in both rats exposed to the forced swim test, and in the tail suspension test in mice [73,76,77]. Both URB597 and AM404 induce anxiolytic-like responses and inhibit stress-induced corticosterone release. However, some remarkable discrepant findings regarding these two compounds have been reported. URB597 administration produced anxiolytic-like effects and an inhibition of stress-induced corticosterone release in a linear, dose-dependent manner, while AM404-induced effects disappeared by increasing the drug dose [78,79]. URB597 and AM404 magnify endocannabinoid signalling through blockade of AEA deactivation, although through different mechanisms. The non-selectivity of AM404, that also activates TRPV1 receptors [80], may account, at least in part, for these different pharmacological profiles.

Even though the pharmacological normalization of the ECS may provide a suitable therapeutic strategy in disease management, a note of caution is always required when extrapolating data from animal studies to human diseases, particularly when considering complex conditions frequently chronic, such as anxiety and depression. In this sense, the studies that are probably more clinically relevant are those that suggest a protective function of CB1R against the consequences of stress and in the adaptation to new stressful environmental conditions [3,5,47,81], which both play a major role in human affective disorders.

5.3. Cannabis, Dependence and Psychopathology

Recent studies have unequivocally documented the occurrence of a cannabis withdrawal syndrome against the widespread belief that cannabis does not produce dependence. Delta-9-tetrahydrocannabinol (THC), the major psycho-tropic constituent of cannabis, has been demonstrated to have reinforcing properties in non-human primates, and a withdrawal syndrome following abstinence from the drug has been described in humans [82-84]. This cannabis withdrawal syndrome seems to be characterized by craving, irritability, anxiety, depressed mood, decreased appetite and sleep difficulties [85]. Furthermore, prolonged cannabis consumption and cannabis withdrawal have been associated with depression [86]. In this regard, a variety of potential treatments have been examined, mainly anxiolytic and antidepressant drugs [87,88]. Actually, inhibitors of AEA deactivation have arisen as a putative medicine for cannabis dependence [89].

Several lines of evidence support an association between an altered ECS and the pathogenesis of schizophrenia. On the one hand, increases in CB1R expression have been found in the prefrontal [90] and cingulate cortex [91] of schizophrenic patients. Elevated levels of AEA have been detected in the cerebrospinal fluid (CSF) of schizophrenics [92-94]; and a negative correlation between CSF-AEA and psychotic symptoms has been reported, thus suggesting that an elevated endocannabinoid tone could serve as an adaptive response to the disease state [92]. On the other hand, there is now evidence demonstrating an association between increased rates of cannabis use and new cases of schizophrenia (see for review [4,95,96]). Cannabis is one of the most abused drugs among teenagers and the maturational processes that occur during adolescence are likely to confer this age group a higher risk of suffering from adverse consequences of cannabinoid exposure. In addition to cannabis dependence, these deleterious consequences may include lasting effects on emotionality and cognition, increased risk for neuropsychiatric disorders and consumption of other drugs of abuse such as nicotine, alcohol and psychostimulants [59,97]. Moreover, cannabis-using patients experience more positive symptoms and frequency of relapse and hospitalization and respond poorly to antipsychotic medication [95,98,99]. Consequently, educational, psychological and social interventions aimed at reducing cannabis use should be promoted so as to reduce dependence and mental health risks in society.

6. CONCLUDING REMARKS AND FUTURE DIRECTIONS

Herein discussed data shall contribute to understand the relevance of a normative endocannabinoid system in psychological homeostasis. CB1R blockade and chronic cannabis consumption support the hypothesis that either deficiency or excess in ECS activation may be associated with or result in anxiety, depressive and psychotic disorders (see Fig. **2**). An increasing body of evidence points to the existence of an intrinsic endocannabinoid tone that may control anxiety levels under basal situations while promoting recovery and/or adaptation to stressful situations, or to their recall. Given the major neuromodulatory role of the ECS, control of emotional responses may critically depend on functional interactions with other monoaminergic and peptidergic systems also involved in the regulation of emotional responses. However, the involvement of the ECS in the regulation of anxiety and its participation in the modulation of behavioural and physiological responses to aversive situations has other obvious implications [1,21-23,81,100]. Disequilibrium or malfunctioning of the ECS might contribute to the aetiology of several psychopathologies, including anxiety-related disorders, depression, psychosis, and drug abuse [3-6]. On contrast, enhancement of endocannabinoid signalling has achieved promising anxiolytic and antidepressant-like effects in a

Table 1. Emotional-Related Effects of Principle Anandamide Deactivation Inhibitors (URB597 and AM404) in Rodents Behavioral Tests for the Evaluation of Anxiety and Depressive-Like Drug Activity

Paradigm	Animal	Drug (mg/kg)	Time Prior Testing	Effect	Reference
Isolation-induced ultrasonic vocalization test	Wistar rat (pnd 10)	URB597 - 0.1	30 min.	↓ Ultrasonic vocalizations	[69]
Zero-maze	Wistar rats	URB597 - 0.1	30 min.	↑ time in open compartment	
Ligh/Dark test	BALB/c mice	AM404 – 1 and 4	30 min.	No effects	[75]
	Sprague-Dawley rats	URB597- 0.1 and 0.3 (1^{st} and 2^{nd} days)	40 min.	↑ time in the light	[73]
		URB597- 0.1 and 0.3 (3^{rd} day)	40 min.	No effects	
	C57 mice	URB597- 1	2h	No effects	[101]
Elevated plus-maze	ICR mice	URB597- 0.1 and 0.3	30 min.	↑ time and entries in open arms	[78]
	Sprague-Dawley rats	AM404 - 0.015	60 min.	—	[74]
		AM404 - 0.75-1.25		↑ time in open arms	
	C57 mice	URB597- 0.1, 1 and 10	30 min.	No effects	[102]
	ICR mice	URB597- 0.1, 1 and 10	30 min.	No effects	
	ICR mice	URB597- 0.1, 1 and 10	30 min.	No effects	
			120 min.	↑ time in open arms (modified EPM according to [78])	
	C57 mice	URB597- 1	2h	↑ time and entries in open arms	[101]
	C57 mice	URB597- 0.1 and 0.5	30 min.	No effects	[103]
		URB597- 1		↑ time and entries in open arms	
	Swiss mice	URB597- 0.1 and 0.5 (7 days)		No effects	
		URB597- 1 (7 days)		↑ time and entries in open arms	

(Table 1. Cont….)

Paradigm	Animal	Drug (mg/kg)	Time Prior Testing	Effect	Reference
Forced swim test	Long-Evans rats	AM404 - 5 (3 times)	23.5, 5 and 1h	↓ immobility	[104]
	Wistar rats	URB597- 0.1 (4 days)	2h	↓ immobility ↑ swimming	[76]
	Wistar rats	URB597- 0.1 and 0.3	40 min.	↓ immobility	[77]
Tail suspension test	C57BL-6 mice	URB597- 0.03 (4 days)	2h	No effects	[76]
		URB597- 0.1 and 0.3 (4 days)	2h	↓ immobility	
	C57 mice	URB597- 0.1-10	30 min.	No effects	[102]
	C57 mice	URB597- 0.1	30 min.	↓ immobility (modified test according to [76])	

In all the experiments above reported drugs were intraperitoneally administered.

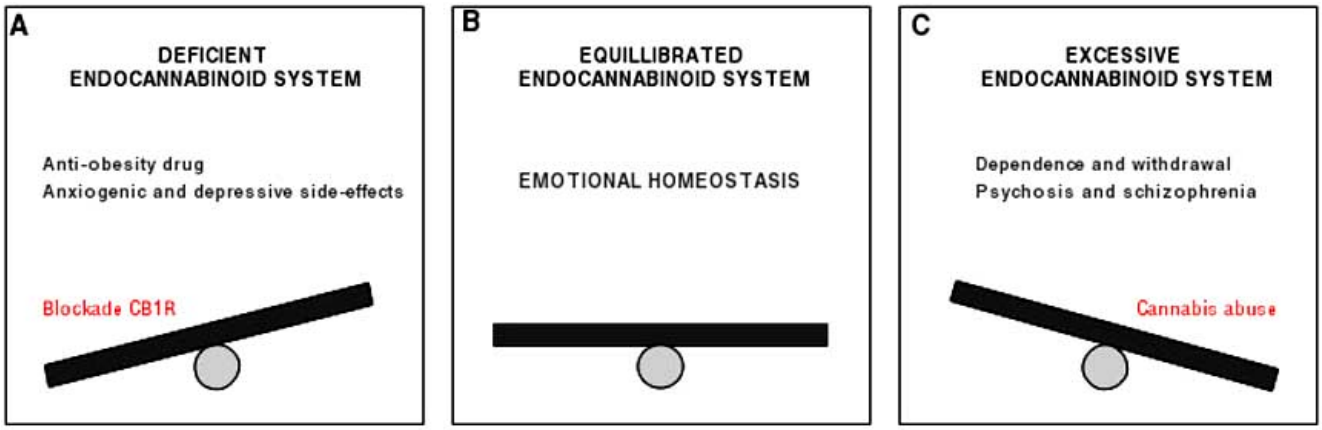

Fig. (2). Hypothetical model representing the harmful consequences of deficient or excessive cannabinoid signalling. On the one hand, deficiencies in the endocannabinoid system (i.e. thorugh blockade of CB1Rs), although representing a promising anti-obesity therapy, may trigger anxiety and depressive-like symptomatology. On the other hand, an excessive and prolonged activation of this endogenous system (i.e. heavy cannabis consumption) may precipitate psychosis and, in some cases, schizophrenia. In spite of the fact that the role of the ECS in emotional regulation is rather complex, it is important to highlight the critical relevance of an equilibrated ECS in maintaining emotionality levels within a safety-physiological range.

variety of animal studies, thus emerging as an attractive strategy for potential therapeutic application [5,7]. As for the opposite strategy, i.e., blockade of CB1Rs, the occurrence of adverse emotional side effects, mainly anxiety and depression, discouraged the application of the CB1R antagonist Rimonabant in the management of obesity associated metabolic disorders and nicotine dependence [50]. In spite of the appealing possibilities of using endocannabinoid modulators as therapeutic strategies, psychiatric side effects and perhaps other undesirable effects are not to be underestimated. Preclinical data and, where appropriate, clinical assays, may contribute to further explore this avenue and to evaluate whether endocannabinoid based medicines would provide any advantage over currently used pharmacological treatments. In any case, a profound investigation of the psychopathological implications of the endocannabinoid system may provide crucial cues about the pathogenesis of several psychiatric diseases as well as for a better understanding of dual pathology, i.e. psychiatric-addiction comorbidity.

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