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The endocannabinoid-alcohol crosstalk: recent advances on a bi-faceted target

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Short Title

Endocannabinoid-alcohol crosstalk

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Abstract

Increasing evidence focuses on the endocannabinoid system as a relevant player in the induction of aberrant synaptic plasticity and related addictive phenotype following chronic excessive alcohol drinking. Besides, the endocannabinoid system is implicated in the pathogenesis of alcoholic liver disease. Interestingly, whereas the involvement of CB1 cannabinoid receptors in alcohol rewarding properties is established, the central and peripheral action of CB2 cannabinoid signalling is still to be elucidated. This review aims at giving the input to deepen knowledge on the role of the endocannabinoid system, highlighting the advancing evidence that suggests that CB1 and CB2 receptors may play opposite roles in the regulation of both the reinforcing properties of alcohol in the brain and the mechanisms responsible for cell injury and inflammation in the hepatic tissue. The manipulation of the endocannabinoid system could represent a bi-faceted strategy to counteract alcohol-related dysfunction in central transmission and liver structural and functional disarrangement.

Key words:

Alcohol; brain; CB1; CB2; endocannabinoids; liver

Introduction

Alcohol use disorder is a chronic relapsing disease characterized by compulsive alcohol seeking and taking[1], loss of control in limiting alcohol intake despite serious negative consequences, and recurring episodes of abstinence and relapse.[2]

According to the World Health Organization Report on Alcohol and Health (2011), alcohol abuse is responsible for at least 60 major types of systemic diseases,[3] such as gut-derived inflammation[4], increased risk of colorectal cancer[5,6] cardiovascular and hearth disease.[7,8] However, the liver has been for long time considered the major victim of the harmful use of alcohol, since it is the main organ responsible for metabolizing alcohol. Several hypotheses have been advanced to explain the pathogenic mechanisms of alcohol-related liver disease, as well as of the aberrant functioning of the brain of alcoholics.[9-11] Accordingly, it would be overambitious, and out of our aim, to elucidate how neurotransmitters and modulators are affected by alcohol, contributing, in turn, to its consequences. Rather, this review will focus on the endocannabinoid system (ECS), as a common critical player in mediating both synaptic neuroadaptation and liver architectural distortion that result from excessive alcohol exposure. Indeed, the ECS is implicated in the regulation of a range of physiological processes and pathological conditions both at the central level and in periphery,[12-14] including neural development, synaptic plasticity, pain, emotionality, immune function, metabolism and energy homeostasis.[15-22] Moreover, if endocannabinoid(s) (eCB) involvement in the central effects of alcohol has been largely reported,[23,24] some evidence has appeared about the role eCB play in mediating alcohol activity in the liver. This is a critical issue, because the identification of a common target accountable for alcohol-related brain and liver disease could pave the way for new therapeutic strategies.

Results

ECS-mediated Alcohol reinforcing effects

Alcohol produces its effects through actions on multiple brain circuits and involves neuroadaptive changes not only in adulthood but especially in critical periods of development.[22,25-31]

The past two decades of clinical research, as well as data derived from preclinical models of alcohol addiction, point to the glutamatergic and the GABAergic systems as the main target of alcohol activity in the mesocorticolimbic system,[32-38] where alcohol intake results in increased dopamine (DA) release from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Notwithstanding, alcohol's mechanism of action at the molecular level is fairly unknown. Some evidence of a direct effect on the dopaminergic system came from findings on the role played by acetaldehyde (ACD), its first oxidative metabolite. Indeed, ACD directly affects dopaminergic neurotransmission, increasing neuronal firing in the VTA, and stimulating DA release in the NAc shell.[21,39-41] Moreover, ACD is able to induce and maintain an addictive-behavior in which seeking and relapse are modulated by the eCB[12,42] so that ACD is believed to play a primary role in the "first-hit" of alcohol reinforcement and in the induction of relapse.[21,22,39,43-46] Indeed, the ECS[21] contributes to alcohol's rewarding effect, and to the occurrence of an addictive phenotype by fine tuning synaptic transmission: the eCB in fact, are key activity-dependent messengers that, by short and long-term decreases in synaptic transmission, regulate glutamatergic and GABAergic synapses. Long-term depression (LTD) is the best-characterized and widespread form of eCB mediated long-term synaptic plasticity. Because behavioural adaptations rely on changes in synaptic strength, the cannabinoid receptor type 1 (CB_1 receptor)-activated-LTD[17,47] represents a fundamental mechanism for making long-term changes to neural circuits and behaviour. Notably, eCB induce synaptic plasticity in the VTA: CB_1 receptor-induced LTD of excitatory inputs to VTA GABA interneurons is basically mediated by 2-arachidonylglycerol (2-AG), which is formed postsynaptically via glutamate metabotropic receptor activation.[48,49] Alcohol itself can induce an enhancement of CB_1 receptor-

dependent LTD by increasing eCB levels: as a matter of fact, alcohol is able to activate phospholipase A2 (PLA2), promote the synthesis of cannabinoid ligands arachidonylethanolamine (AEA) and 2-AG in neuronal cells, hinder fatty-acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) activity.[50,51] This potentiation of CB_1 receptor-dependent LTD can consequently promote an enhancement in VTA dopaminergic signalling and result in increased alcohol rewarding effects. Interestingly, cue-induced alcohol craving can also result in a marked elevation of circulating levels of AEA,[52] suggesting that AEA mobilization in peripheral tissues may be a marker of activation of the ECS in the brain.

The involvement of CB_1 receptors in alcohol rewarding effect is further confirmed by studies showing that the administration of CB_1 receptor agonists increases alcohol consumption in animal models of addiction.[53,54] In particular, the administration of WIN 55,212-2, a synthetic non-selective CB_1 receptor agonist, causes a long-lasting increase in alcohol consumption during the re-exposure to alcohol in an animal model of relapse-like drinking[55] and is able to modulate binge-like alcohol intake in male mice.[56] Moreover, CB_1 receptor agonist CP-55,940 increases alcohol preference in a two-bottle choice paradigm[57] and alcohol-maintained responses in a progressive ratio paradigm.[58] On the other hand, the systemic administration of CB_1 receptor antagonists, such as SR141716A (rimonabant) and AM251, is able to decrease voluntary alcohol intake in naïve-[59,60] and in alcohol-preferring rats and mice.[61,62] CB_1 receptor antagonists can also reduce alcohol-seeking behaviour in a rat model of relapse[63] and decrease binge alcohol and sucrose consumption in adolescent and adult mice.[54] Overall, the role of CB_1 receptors on alcohol reinforcing properties has been mainly unveiled by research on CB_1 receptor knockout mice, whose receptor deletion significantly reduces alcohol preference[64] and decreases its intake and conditioned place preference.[65,66,67] Interestingly, Hungund et al. showed that mice lacking CB_1 receptors consume less alcohol than their wild-type counterparts and do not display alcohol-induced increase in DA release in the NAc.[65]

In contrast, Linsenbardt and Bohem[56] showed a decrease in alcohol intake in male mice induced by high doses of WIN 55,212-2 in the first 30 minutes of Drinking In the Dark; accordingly, Cippitelli et al.[68] reported a decline in alcohol self-administration in Wistar rats, as a consequence of CB_1 receptors stimulation. However, it cannot be ruled out that the reduction in fluid consumption may be due to the motor inhibition component of the CB_1 receptor agonism. Moreover, inconsistent results came from studies employing cannabinoid receptor deletion: indeed, CB_1 receptor-deficient mice initially show a higher alcohol preference and alcohol intake than wild type animals, while, after the first week, CB_1 receptor deficient mice display a similar drinking pattern as wild type, suggesting that cannabinoid modulation of the rewarding stimuli plays a fundamental role in alcohol approach.[69]

Besides the involvement of classical neurotransmitters, the eCB's modulation of the response to alcohol involves the recruitment of reward/stress-related neuropeptides - such as CRH and Neuropeptide Y (NPY) – which play a critical role in the development of addiction.[70,71] Indeed, recent findings show that rat exposure to high concentration of ACD induces an increase in eCB transmission and this results in a downregulation of NPY expression and the occurrence of withdrawal symptoms following ACD suspension.[21,22] Moreover, the study showed that CB_1 receptor blockade by the antagonist/inverse agonist AM281 is able to increase NPY expression, decrease ACD seeking-behaviour and boost homeostatic functional recovery.[21,22] This is in accordance with AEA and 2-AG effect on CB_1 receptors on glutamate terminals, and the resulting decrease in glutamate release and downregulation in NPY mRNA levels[72,73]. Overall CB_1 receptor signalling in the mesocorticolimbic system is a fundamental prerequisite for the expression of motivation to seek rewarding stimuli (Figure 1a).

CB_2 receptors: A Novel Target for Addiction

When cannabinoid receptors type 2 (CB_2 receptor) were first cloned, they were tagged as peripheral receptors.[74] However, recent studies suggest that CB_2 receptors are expressed in the nervous system – certainly in activated microglia and in some neuron subsets.[75]

Indeed, CB_2 receptors have been recently involved in synaptic plasticity,[76,77] and latest studies investigated the role of CB_2 receptors in models of alcohol, nicotine and cocaine addiction[78-80] suggesting a role, not well defined yet, for CB_2 receptors in the modulation of drug reward-related behaviours.[81]

A recent study by Liu and colleagues provided a powerful new genetic tool for elucidating the functional role of CB_2 receptors in the CNS, by their selective deletion in dopaminergic neurons.[82] They demonstrated that CB_2 receptors put a "brake" on the classical locomotor activation that follows dopaminergic stimulation, since their conditional deletion in DAT positive midbrain dopaminergic neurons in DAT-Cnr2 cKO mice enhances psychomotor behavior. Traditionally CB_1 receptor agonism was associated with the tetrad effects in mice, producing the characteristic profile of suppression of locomotion, antinociception, hypothermia and catalepsy. This notion had been supported using data from radioligand binding and in-vivo behavioral assays that lacked sensitivity and cell-type specific deletion of the CCB_2 receptors. The dopaminergic neuron-specific deletion of the CB_2 receptors allowed providing evidence of the contribution of both CB_1 receptors and CB_2 receptors in the cannabinoid tetrad task. This is consistent with preclinical studies showing that selective CB_2 receptor agonists exert an attenuation of neuropathic pain pathways in rodent pain models[83,84] and suggest that CB_2 receptors may contribute to the physical effect resulting from ECS direct (or alcohol-evoked) activation.

Both CB_1 receptor and CB_2 receptor pathways show great diversity and complexity with distinct pre- or post-synaptic distribution patterns where they may work cooperatively or in opposition to modulate the effects of cannabinoid and eCB in diverse brain areas. Thus, activation of postsynaptic CB_2 receptors in relevant brain areas[85,86,87] supports the inhibition of VTA DA neuronal firing by CB_2 receptors. This may be associated with the resistance of the DAT-Cnr2 cKO to the induction of conditioned place preference caused by alcohol. Presynaptic CB_1 receptors indeed are not expressed in VTA DA neurons and the major endocannabinoid function in DA neuron in the VTA is mediated by postsynaptic CB_2 receptors that play an inhibitory role with respect to alcohol's rewarding effect.

However, the DAT-Cnr2 cKO mice showed an increased sensitivity to the rewarding effects of cocaine and this contributes to the existing evidence regarding the role of CB_2 receptors in the addicted properties of this drug. Notably, the activation of brain CB_2 receptors inhibits cocaine self-administration, and increases in locomotion, DA neuronal firing and extracellular DA release in the NAc in mice.[88] This inhibitory effect is reversed by pharmacological blockade of CB_2 receptors by AM630 or is absent in CB_2 receptor knockout mice, suggesting specificity in CB_2 receptor-mediated effect. This apparent discrepancy, together with the modulating effect of alcohol reinforcing properties, illustrates the complexity of CB_2 receptors involvement in addiction.

Interestingly, CB_2 receptors are expressed not only on VTA dopaminergic neurons,[88,89] but also in the hippocampus and substantia nigra where they occupy a postsynaptic position[90]. These localizations might be associated with psychomotor and cognitive specific functions in balance with the activation of presynaptic CB_1 receptors.

Given that dysfunction in DA signalling is a major abnormality in psychiatric and neurological disorders,[91] these studies provide a basis for further analysis of the role of CB_2 receptors in the etiology of central disorders associated with DA dysregulation and for the development of drugs that selectively target eCB receptors (Figure 1a).

ECS-mediated effects in Alcoholic Liver Disease

The ECS and its receptors have emerged as major regulators of several pathophysiological mechanisms responsible for cell injury and inflammatory response that are associated with chronic liver disease progression.[92-94] Indeed, under physiological conditions, both hepatocytes and non-parenchymal cells (i.e. Kupffer) are able to produce eCB, but the basal expression of CB_1 receptors and CB_2 receptors in the adult healthy liver is low or even absent.[95,96] Notably, eCB synthesis and the hepatic expression of both cannabinoid receptors are upregulated during chronic liver damage.[92]

Studies applying genetic or pharmacological inactivation of cannabinoid receptors showed that CB_1 receptors and CB_2 receptors exert opposite effects on fibrogenesis: CB_1 receptors have profibrogenic effects,[95] while CB_2 receptors can inhibit or reverse hepatic fibrogenesis and exert anti-inflammatory effects,[97] thus protecting from liver damage progression (Figure 1b).[14,98] Notably, alcohol intake increases the hepatic expression of CB_1 receptors and, in activated stellate cells, it upregulates 2-AG, which, in turn, stimulates the deposition of fat in neighbouring hepatocytes by binding overly expressed CB_1 receptors.[99] On the other hand, CB_1 receptor knockout mice are resistant to the steatogenic and fibrogenic effects of alcohol, while CB_2 receptor knockout mice display increased collagen deposition, liver fat, and enhanced inflammatory scores.[95-100]

Accordingly, selective antagonists and agonists of respectively CB_1 receptors and CB_2 receptors, may attenuate the development of alcoholic liver disease.[99,101,102] In particular, CB_1 receptor blockade by rimonabant and AM6545 induces partial regression of fibrosis and steatosis, restoring liver architecture.[103] Rimonabant administration to wild-type mice, as well as the genetic inactivation of CB_1 receptors are both associated with a significant reduction in fibrosis progression[95], reduced hepatic expression of the profibrogenic cytokines and decreased number of fibrogenic cells. Antifibrogenic properties of the CB_1 receptor-selective antagonist were ascribed to the antiproliferative and apoptotic properties of the compound in hepatic myofibroblasts.[104] Notwithstanding the beneficial effects of rimonabant in ameliorating hepatic fibrosis, the compound

was withdrawn from the market because of its psychotropic side effects, such as mood disorders that, in susceptible individuals, could also lead to major depression with high suicidal risk.[105] However novel compounds are currently under investigation in preclinical settings,able to modulate the eCB signalling while sparing the brain from toxicity.

Indeed, Denaës and colleagues recently demonstrated that CB_2 receptors agonism can protect from alcoholic liver disease by inhibiting hepatic inflammation and steatosis in Kupffer cell through an autophagy-dependent pathway.[102] Moreover, CB_2 receptor agonism can facilitate hepatic regeneration, as well as normalization of serum liver enzymes, triglycerides, free fatty acids, and cholesterol.[103] In alcohol-exposed mice, endogenous or exogenous activation of CB_2 receptors prevents Kupffer cells from switching into a pro-inflammatory phenotype and hepatocytes from accumulating triglycerides.[97] In vitro activation of CB_2 receptors regulates macrophage polarization by preventing pro-inflammatory responses and inducing polarization towards an anti-inflammatory phenotype.[97] Because CB_2 receptors are not expressed in hepatocytes,[106] the antisteatogenic signal could originate from Kupffer cells.

Overall these data pinpoint on the CB_1 receptor signalling pathway as a novel paradigm to explain the molecular mechanisms underlying alcohol-induced liver damage. Moreover, hepatic CB_2 receptors emerge as protective targets not only for their antifibrogenic properties but also for promoting hepatocyte survival and regeneration.

The rising availability and use of legal cannabis by individuals who also consume alcohol, make the assessment of cannabis impact on alcohol-related pathologies mandatory. Daily cannabis smoking has been pointed as an additive factor in the evolution of ALD.[5] However, the synergistic effects of cannabis and alcohol on liver disease progression remains unclear and further research in this field is highly needed.

Discussion

The rapidly advancing evidence on the neurobiology of the ECS suggests that CB_1 receptors and CB_2 receptors may work independently and/or cooperatively to regulate diverse physiological functions and pathogenetic processes. Based on the present findings, we support the hypothesis of CB_1 receptors and CB_2 receptors playing opposite roles in the regulation of the reinforcing properties of alcohol that, in turn, is related to stimulation of eCB transmission at central and peripheral levels. The ECS regulates both pro- and antifibrogenic responses in the liver with CB_1 receptors exerting profibrogenic effects and CB_2 receptors inhibiting hepatic fibrogenesis and exerting anti-inflammatory effects. Overall ECS manipulation could represent a common strategy to reduce the incentive reinforcing properties of alcohol and to recover liver architecture and functional disarrangement. Further elucidation on the eCB-alcohol-crosstalk, will help finding specific agents able to respond to complex therapeutic demand.

Methods

The literature search targeted evidence-based guidelines, evidence-based summaries, systematic reviews and recent experimental research on alcohol and endocannabinoid central, peripheral and behavioural effects.

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Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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