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Interactions Between Alcohol and the Endocannabinoid System

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Abstract

Endocannabinoids are lipid mediators that interact with the same cannabinoid receptors that recognize Δ^9-tetrahydrocannabinol (THC), the psychoactive constituent of marijuana, to induce similar effects in the brain and periphery. Alcohol and THC are both addictive substances whose acute use elicits rewarding effects that can lead to chronic and compulsive use via engaging similar signaling pathways in the brain. In the liver, both alcohol and endocannabinoids activate lipogenic gene expression leading to fatty liver disease. This review focuses on evidence accumulated over the last two decades to indicate that both the addictive neural effects of ethanol and its organ toxic effects in the liver and elsewhere are mediated, to a large extent, by endocannabinoids signaling via cannabinoid-1 receptors (CB_1R). The therapeutic potential of CB_1R blockade, globally or in peripheral tissues only is also discussed.

Keywords

endocannabinoids; cannabinoid receptors; alcohol drinking behavior; alcoholic liver disease

THE ENDOCANNABINOID SYSTEM (ECS)

In recent decades, there has been growing interest in the biology of cannabinoids, fueled in part by the potential health benefits of marijuana and its different constituents but also by the discovery of endogenous cannabinoids (endocannabinoids) and their role in biological functions in health and disease. The story of endocannabinoids started in the late 1980's with the discovery that the biological effects of Δ^9-tetrahydrocannabinol (THC), the psychoactive component of marijuana, are mediated by G-protein-coupled receptors (GPCR) that negatively regulate adenylate cyclase (Devane et al., 1988). This was contrary to the earlier prevailing notion that THC, a highly lipophilic compound, acts by non-specific perturbation of membrane lipids. Within a few years, two such GPCR were identified by molecular cloning: the cannabinoid-1 receptor (CB_1R) (Matsuda et al., 1990), which is the most abundant receptor in the mammalian brain but is also expressed at much lower yet functionally relevant levels in most peripheral tissues and the CB_2R (Munro et al., 1993), which is expressed predominantly, although not exclusively, in cells of the immune

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CONFLICT OF INTEREST

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and hematopoietic systems. The presence of receptors in mammalian tissues for a plant-derived substance triggered a search for endogenous ligands. In the 1990's, a number of endogenous, arachidonic acid-containing lipids were found to bind to and activate CB_1R and CB_2R with high affinity, the two most widely studied ones being anandamide (arachidonoyl ethanolamide) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG)(Mechoulam et al., 1995; Sugiura et al., 1995), collectively called endocannabinoids. Although other GPCR also recognize some cannabinoids and related endogenous lipids (GPR18, GPR55, GPR119, reviewed in (Irving et al., 2017)), the role of these receptors in the biology of endocannabinoids is less well understood. Endocannabinoids, primarily anandamide, also interact with other targets including Transient Receptor Potential (TRP) channels and Peroxisome Proliferation-Activated Receptors (PPAR), albeit with lower affinity than their affinity for cannabinoid receptors. These interactions and their possible physio-pathological implications have been recently reviewed (Iannotti et al., 2016).

Endocannabinoids are generated on demand in the cell membrane from membrane phospholipid precursors, in response to a rise in intracellular calcium or activation of metabotropic GPCR, including metabotropic glutamate receptors. The immediate membrane precursor of anandamide is N-arachidonoyl phosphatidylethanolamine (NAPE), from which it can be generated by multiple parallel pathways including NAPE-specific PLD, as reviewed recently (Hussain et al., 2017), whereas the metabolism of anandamide is catalyzed primarily by fatty acid amide hydrolase (FAAH)(Cravatt et al., 1996). In the brain, 2-AG is generated from diacylglycerol predominantly via diacylglycerol lipase α (DAGLα), whereas in the liver the same process is catalyzed by DAGLβ (Bisogno et al., 2003). Metabolic degradation of 2-AG is catalyzed primarily by monoacylglycerol lipase (MAGL)(Labar et al., 2010), with minor contributions by α/β hydrolases ABHD6 and ABHD12 (Blankman et al., 2007). The endocannabinoids, their cognate receptors and the enzymatic machinery involved in their biosynthesis and degradation constitute the endocannabinoid system (ECS).

During the twenty-five or so years that have passed since their discovery, endocannabinoids have been implicated in the regulation of a growing number of biological functions both in the brain and in the periphery. Such studies were enabled by the introduction of highly selective and potent inhibitors of cannabinoid receptors (Rinaldi-Carmona et al., 1994; Rinaldi-Carmona et al., 1998) and of the enzymes involved in their biosynthesis (Baggelaar et al., 2015) and degradation (Fegley et al., 2005; Long et al., 2009). Additional critical tools for such studies were mice deficient in CB_1R (Ledent et al., 1999; Zimmer et al., 1999), FAAH (Cravatt et al., 2001), MAGL (Taschler et al., 2011) and DAGLα and β (Gao et al., 2010). In the CNS, endocannabinoids had been identified as the long sought retrograde transmitters (Alger, 2002) that are generated in postsynaptic cells and act at CB_1R located on presynaptic terminals (Katona et al., 1999; Wilson et al., 2001) to mediate some forms of synaptic plasticity (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Particularly relevant to the present review, endocannabinoids acting via CB_1R are be obligatory components of the mesolimbic dopaminergic reward pathway (Solinas et al., 2008) that mediates both natural and drug reward (Gessa et al., 1998), including the rewarding effects of alcohol (Hungund et al., 2003). The ECS is also present and functional in peripheral tissues (Maccarrone et al., 2015), including the liver where activation of CB_1R promotes *de novo* lipogenesis (Osei-Hyiaman et al., 2005) and mediates the similar action of

ethanol (Jeong et al., 2008). Here we review progress in our understanding of the role of the ECS in both ethanol seeking behavior and in ethanol-induced organ damage, with a focus on recent advances. For many important earlier studies, the reader is referred to excellent recent reviews of this topic (Gianessi et al., 2019; Henderson-Redmond et al., 2016; Maccioni et al., 2010; Pava and Woodward, 2012; Siegmund, 2010; Talani and Lovinger, 2015).

SIMILARITIES BETWEEN ALCOHOL AND CANNABIS USE DISORDERS

Well before the ECS or molecular targets for alcohol were discovered and both ethanol and cannabis were thought to act by non-specific perturbation of membrane lipids, intriguing similarities between alcohol use disorders (AUD) and cannabis use disorders (CUD) had been noted. At the epidemiological level, the proportion of the population that becomes dependent on ethanol or cannabis is the highest in adolescents and gradually declines thereafter for both substances (Grant and Dawson, 1997; Stinson et al., 2006). Furthermore, there is a high degree of comorbidity between AUD and CUD, which is strongest in adolescence (Duncan et al., 2015). Probably the closest indication of a functional link between ethanol and cannabis in early studies was evidence for cross-tolerance between their respective behavioral and neurocognitive effects (reviewed in (Pava and Woodward, 2012).

An age-dependent decline in alcohol preference has also been noted in rodent models of drinking behavior. Ethanol preference and intake by the ethanol preferring C57Bl6J mouse strain was twice as high in young (6–10 wk) than old (26–48 wk) male mice (Wang et al., 2003) and a similar large difference, although at lower levels of ethanol preference, had been noted in the alcohol aversive BALB/c mouse strain (Kakihana and McClearn, 1963). This indicated that the age dependence of alcohol preference manifests not only longitudinally in a given strain but also across strains with genetically determined differences in alcohol preference. This age-dependent decline in alcohol preference was linked to the ECS by the observation that rimonabant treatment of young C57Bl/6J mice reduced their alcohol preference to that seen in older animals, but was ineffective in modifying alcohol preference in the latter or in CB_1R knockout mice, which displayed similar low alcohol preference in both age groups (Wang et al., 2003). Young and old mice had similar brain levels of CB_1R or endocannabinoids but there was an age-dependent decline in CB_1R coupling as measured by GTPγS binding in the limbic forebrain, a group of subcortical structures including the hippocampus, hypothalamus and amygdala involved in the control of emotions and motivational behavior, which may account for the observed age-dependent loss of a CB_1R-mediated component in drinking preference (Wang et al., 2003). An analogous finding was the increased CB_1R coupling efficiency found in the striatum of alcohol-preferring C57Bl/6J versus alcohol aversive DBA/2J mice (Vinod et al., 2008b). Selectively bred rodent lines have been used to explore the genetic determinants of alcohol-seeking behavior, as reviewed recently (Spence et al., 2009).

Similar to the age-dependence of the effect of rimonabant on drinking, pre-pubertal male C57Bl/6J mice tested at postnatal day (PD) 21 in a binge drinking paradigm had higher alcohol intake than adult (PD 63) mice and the CB_1R antagonist AM-251 was more potent in reducing drinking in the younger compared to the older group (Agoglia et al.,

2016). Studies with agonists also point to an age-dependent decline in cannabinoid/ethanol interactions. Treatment of pubertal (postnatal day 40) or adult Wistar rats (PD 100) with WIN 55,212–2 caused heightened anxiety and increased alcohol preference in the young but not the adult animals, which was linked to a similar age-dependent, WIN 55,212–2-induced increase in the expression of the NR1 subunit of the NMDA receptor and its regulatory protein CC-Homer (Klugmann et al., 2011), suggesting a role of the glycine binding site of the NMDA receptor in cannabinoid-induced drinking. Indeed, the NMDA/glycine receptor antagonist L-701 was reported to inhibit WIN 55,212–2-induced alcohol drinking (Alen et al., 2009). WIN 55,212–2 similarly increased anxiety and alcohol preference in early adolescent CD1 mice (Frontera et al., 2018).

There is also a close similarity between the mechanisms and anatomical substrates involved in ethanol and cannabinoid reward, with the mesolimbic dopaminergic reward pathway being involved in both (Cheer et al., 2007). In freely moving rats, i.p. injections of low doses of ethanol caused dose-dependent release of dopamine in the dopaminergic projection area of the nucleus accumbens (NAc) with less release in the subcortical dorsal caudate nucleus, as measured by *in vivo* microdialysis (Di Chiara and Imperato, 1988). The increase in ethanol-induced dopamine release in the NAc was greater in alcohol preferring than in alcohol-avoiding rats (Bustamante et al., 2008). Interestingly, salsolinol, an endogenous adduct of dopamine and the alcohol metabolite acetaldehyde that is self-administered into the ventral tegmental area (VTA) where it induces a strong place preference, also activate VTA dopaminergic neurons (Xie et al., 2013). Cannabinoids produce similar effects: CB_1R activation by systemic administration of THC or the synthetic cannabinoid WIN55,212–2 to rats elicited dopamine release in the NAc shell as detected by microdialysis (Tanda et al., 1997), and increased the spontaneous firing rate of dopamine neurons projecting to the NAc (Gessa et al., 1998). VTA dopaminergic neurons do not express CB_1R and the increase in DA release by cannabinoids is believed to be mediated by CB_1R on inhibitory GABAergic interneurons, resulting in disinhibition of VTA dopaminergic neurons and enhanced dopamine release in the NAc (Cheer et al., 2004; Lupica et al., 2004). However, the modulation of dopamine release in the NAc by endocannabinoids is more complex, as activation of CB_1R on prefronto-cortical glutamatergic terminals in the NAc reduces the release of glutamate and, consequently, dopamine, which may be relevant for limiting the rewarding effects of acute drug exposure, and may play a role in relapse to drug seeking behavior (Mateo et al., 2017). Whether ethanol has a similar action, has not yet been observed.

ETHANOL EFFECTS ON ENDOCANNABINOID PRODUCTION AND CB_1R EXPRESSION

There is fairly consistent evidence that either short-term or chronic exposure of various cultured neuronal cell types to ethanol upregulates the production of both anandamide and 2-AG in a calcium-dependent manner (Basavarajappa and Hungund, 1999; Basavarajappa et al., 2008; Basavarajappa et al., 2000) and acute exposure of alcohol also upregulates anandamide *in vivo* in the NAc of rats (Ceccarini et al., 2013) or in the hippocampus and cortex of immature mice (Subbanna et al., 2013). Whereas chronic exposure to alcohol

can also increase endocannabinoid levels, as found for 2-AG in the dorsolateral striatum (DLS)(DePoy et al., 2013), this can result in the downregulation of CB_1R expression in the DLS as well as in other brain regions (Ceccarini et al., 2014; Hirvonen et al., 2013; Ortiz et al., 2004), which may be one of the mechanisms that underlies the negative affect associated with chronic alcoholism.

The striatum is involved in the regulation of voluntary behavior and cognition, which are impaired by chronic alcohol consumption. The mechanisms underlying the effects of alcohol may involve changes in synaptic plasticity: low concentrations of alcohol were found to inhibit NMDA receptor-mediated long-term potentiation (LTP), which may be involved in the acute intoxicating effects, whereas higher concentrations of ethanol (50 mM) promoted long-term depression (LTD) in the dorsomedial striatum, a brain region implicated in the learning and selection of goal-directed behaviors (Yin et al., 2007). There is evidence that these effects of ethanol are mediated via interference with endocannabinoid activation of CB_1R. In the dorsolateral striatum (DLS), ethanol inhibits CB_1R-mediated long-lasting disinhibition of striatal output and also reduces LTD, induced by different stimulation parameters, at a site downstream from CB_1R activation (Clarke and Adermark, 2010). In another study, acute exposure of DLS slices to 50 mM ethanol depressed striatal output measured by population spike amplitude, whereas washout of ethanol had the opposite effect, i.e. facilitation, which is mediated via cholinergic interneurons (Adermark et al., 2011a). These findings reveal complex effects of ethanol on synaptic plasticity and the neural circuits involved, although they do not directly address the role of endocannabinoids. The role of endocannabinoids and CB_1R in the DLS was explored in a more recent study, in which chronic intermittent exposure of mice to alcohol vapor resulted in the absence of CB_1R-mediated LTD in DLS neurons, which was associated with facilitation of various, DLS-dependent learning paradigms (DePoy et al., 2013).

ENDOCANNABINOID EFFECTS ON ALCOHOL DRINKING BEHAVIOR

There are three lines of evidence supporting the role of the ECS in the regulation of alcohol drinking behavior: a) the effects of direct treatment with a cannabinoid agonist; b) the effects of increased endocannabinoid tone achieved by pharmacological blockade or genetic deletion of endocannabinoid degrading enzymes; and c) the effects of selective cannabinoid receptor antagonists or genetic deletion of these receptors.

a) Agonists.

As for agonist effects, an early study in Wistar rats documented dose-dependent increases in the motivation to drink beer by the potent CB_1R agonist CP 55,940, using an operant drinking paradigm. A similar effect on the intake of a sucrose solution suggested a general effect on appetite for palatable beverages rather than a specific effect on alcohol (Gallate et al., 1999). Using an operant drinking paradigm, the same group found that THC can reinstate beer drinking by rats but, again, the same was true for sucrose intake (McGregor et al., 2005). More specificity was observed in ethanol-preferring sP rats, in which similar doses of CP 55,940 or somewhat higher doses of WIN 55,212–2 selectively increased ethanol but not sucrose drinking (Colombo et al., 2002). Low doses of WIN

55,212–2 administered either systemically (0.5 mg/kg) or into the posterior VTA (0.25 μg/side) similarly increased ethanol intake in the 'drinking in the dark' binge drinking paradigm. This highlighted the role of CB_1R in the pVTA in promoting alcohol drinking presumably by disinhibiting VTA dopaminergic neurons (Linsenbardt and Boehm, 2009), in agreement with findings in alcohol-preferring AA rats (Malinen and Hyytia, 2008). In the same study, higher systemic (1–2 mg/kg) or intra-pVTA doses of WIN 55,212–2 (0.5–2.5 μg/side) suppressed alcohol drinking as well as total water intake, most likely as the result of CB_1R-induced hypomotility. This illustrates a pitfall of using direct acting CB_1R agonists whose behavioral effects may be confounded by their robust display of the 'tetrad' responses that are the hallmarks of CB_1R activation (hypomotility, catalepsy, hypothermia and hypoalgesia), particularly hypomotility and catalepsy.

Endocannabinoids and CB_1R also appear to mediate the inhibitory effect of ethanol on adult hippocampal neurogenesis. Treatment of Wistar rats with a CB_1R agonist or an inhibitor of endocannabinoid degradation mimicked the inhibitory effect of alcohol, whereas treatment with CB_1R antagonists prevented alcohol-induced inhibition of adult hippocampal neurogenesis (Khatri et al., 2018).

b) Inhibitors of endocannabinoid degrading enzymes

In contrast to the direct effect of synthetic agonists, the biological effects of endocannabinoids including their effects on alcohol drinking behavior may be unmasked by preventing their metabolic degradation. As mentioned before, anandamide is primarily metabolized by FAAH whereas 2-AG is degraded mainly by MAGL. Both these enzymes are tonically active, so their pharmacological inhibition or genetic deletion results in large and selective increases in the tissue levels of their respective substrates. Anandamide involvement in alcohol preference was first suggested by findings in C57Bl/6J mice with genetic deletion of FAAH (Basavarajappa et al., 2006). Female wild-type mice had higher alcohol preference and intake than littermate male mice and germline deletion of FAAH further increased alcohol preference and intake in female but not male mice (Basavarajappa et al., 2006). No such sex differences were found in other studies, in which genetic deletion of FAAH in mice on a C57Bl/6J genetic background resulted in increased preference for alcohol but not for sweetened or bitter solutions, as well as reduced sensitivity to the sedative and motor coordination disruptive effects of ethanol in both male and female mice (Blednov et al., 2007; Vinod et al., 2008a). These effects were replicated by the treatment of wild-type mice with the FAAH inhibitor URB597, whereas URB597 treatment of $CB_1R^{-/-}$ mice did not affect the lower baseline alcohol preference of these animals. This supported the role of CB_1R in the increased alcohol preference induced by FAAH inhibition or deletion (Vinod et al., 2008a). Consistent with these findings, alcohol preferring AA rats had decreased expression and activity of FAAH in the prefrontal cortex (PFC), and intra-PFC injections of rimonabant reduced alcohol self-administration, whereas intra-PFC injections of URB597 in non-selected Wistar rats increased alcohol self-administration (Hansson et al., 2007). An association between impaired FAAH activity and increased preference for ethanol drinking was also supported by a study using knock-in mice expressing a mutant FAAH. In human subjects, the C385A single nucleotide polymorphism (SNP) in the FAAH gene was reported to be associated with reduced FAAH expression in peripheral tissues and

increased incidence of problem drug use (Sipe et al., 2002). A knock-in mouse harboring the same variant allele confirmed its effect in reducing FAAH expression and activity, resulting in a decrease in anxiety-like behaviors (Dincheva et al., 2015). Binge-like alcohol intake was significantly higher in the $FAAH^{A/A}$ mutant than in the $FAAH^{C/C}$ controls with no difference in saccharin or sucrose intake, and alcohol intake was dose-dependently inhibited by a CB_1R antagonist (Zhou et al., 2016).

A very different picture emerges from other studies exploring the role of FAAH in the negative affect associated with chronic alcohol use and withdrawal. In a study using Wistar rats and Marchigian Sardinian alcohol preferring (msP) rats, URB597 treatment neither increased voluntary drinking by msP rats nor facilitated operant alcohol self-administration by Wistar rats, even though rimonabant reduced self-administration. URB597 also failed to influence cue- or stress-induced reinstatement, whereas it robustly suppressed withdrawal-induced anxiety (Cippitelli et al., 2008). URB597 treatment was also reported to prevent the alcohol deprivation effect observed when alcohol is reinstated following withdrawal, which was also blocked by rimonabant (Zhou et al., 2017). An analogous finding is the reported effect of the MAGL inhibitor JZL-184 in mitigating the protracted affective disturbance present in alcohol withdrawn mice, which implicates 2-AG in this protective effect (Holleran et al., 2016). Interestingly, the duality in the effects of FAAH inhibition, i.e. promoting the rewarding salience of ethanol versus inhibiting the negative affect of withdrawal, also applies to MAGL inhibition: a 7-day methamphetamine treatment protocol in mice increased alcohol preference, which was inhibited by the CB_1R antagonist AM251 but potentiated by the MAGL inhibitor *N*-arachidonoyl maleimide (Gutierrez-Lopez et al., 2010).

These seemingly conflicting findings could be reconciled within the framework of the Koobian concept of opponent processes of addiction, i.e. euphoria caused by acute alcohol followed by the dysphoria that develops with repeated withdrawals (Koob, 2019). Accordingly, the acute increase in anandamide induced by FAAH inhibitors promotes alcohol reward and thus increases its abuse potential, which could be therapeutically countered by CB_1R antagonists, whereas the negative affect related to alcohol withdrawal may be mitigated by FAAH or MAGL inhibitors by promoting the CB_1R-mediated anti-anxiety actions of anandamide or 2-AG, respectively. This concept gained further support from a CB_1R PET study in which acute exposure to alcohol resulted in a global increase in CB_1R availability in the brain whereas chronic intake in alcoholic patients caused long lasting reductions in CB_1R availability in various brain regions including the ventral striatum, which may underlie the negative affect of prolonged withdrawal and abstinence (Ceccarini et al., 2014). A long-lasting global reduction of CB_1R availability in chronic alcoholics was also documented in another CB_1R PET study (Hirvonen et al., 2013). As a sign of further complexity, the anxiogenic effect of abstinence that followed chronic exposure to alcohol was found to be reduced by rimonabant (Lallemand et al., 2001; Rubio et al., 2008), which stands in contrast to the similar anxiolytic effects of FAAH or MAGL inhibitors.

A possible explanation of this paradox involves relative contribution of activation of CB_1R on GABAergic vs glutamatergic terminals. In the above study by Rubio et al. (2008), ethanol withdrawal was associated with significant reductions in GABA in the PFC,

caudate-putamen, globus pallidus and amygdala, and the reductions were reversed in the first 3 regions by rimonabant, which may underlie rimonabant's anxiolytic effect in this paradigm. Consistently, conditional knockout of CB_1R in cortical glutamatergic neurons abolished the anxiolytic effects of low dose CB_1R agonists, whereas conditional knockout of CB_1R in GABAergic neurons resulted in the loss of the anxiogenic effects of high doses of CB_1R agonists (Rey et al., 2012).

c) Effects of blockade or deletion of CB_1R

The third, and most decisive, argument to support the role of the ECS in mediating alcohol reward comes from studies documenting the effect of pharmacological antagonism or genetic deletion of CB_1R on alcohol preference and intake in different drinking paradigms. Soon after the introduction of rimonabant (SR141716), it was reported that systemic administration of rimonabant or its analog SR147778 reduced voluntary alcohol intake in various rodent models of drinking (Arnone et al., 1997; Colombo et al., 1998; Femenia et al., 2010; Gallate and McGregor, 1999; Lallemand and De Witte, 2006; Lallemand et al., 2001; Wang et al., 2003) and also suppressed the motivational properties of alcohol in both wild-type and alcohol-preferring rodents (Colombo et al., 2004; Dyr et al., 2008; Economidou et al., 2006; Gessa et al., 2005; Malinen and Hyytia, 2008; Serra et al., 2001; Vinod et al., 2008b). Furthermore, rimonabant also attenuated the increased intake in alcohol in response to low doses of morphine, highlighting a functional link between the endogenous opioid system and the ECS in the control of alcohol drinking behavior (Vacca et al., 2002). Similar effects were obtained following localized microinjection of rimonabant into the VTA (Malinen and Hyytia, 2008) and in particular its posterior region (Alvarez-Jaimes et al., 2009), into the nucleus accumbens (NAc) of AA mice (Malinen and Hyytia, 2008) or Wistar rats (Alvarez-Jaimes et al., 2009) and, in some rat lines, into the medial prefrontal cortex (Hansson et al., 2007). The observation of a reduced endocannabinoid-mediated, **d**epolarization-induced **s**uppression of **i**nhibition (DSI) in the VTA of alcohol-preferring sP rats compared to non-preferring sNP rats (Melis et al., 2009) may reflect an underlying increase in the tonic activity of the ECS in the former, which would be reversed by CB_1R blockade. Together, these findings suggest that components of the mesolimbic dopaminergic reward pathway are one of the targets through which CB_1R blockade inhibits alcohol-seeking behavior.

In an *in vivo* microdialysis study, alcohol self-administration in male Wistar rats elicited a selective increase in the extracellular levels of 2-AG but not anandamide in microdyalisates from the NAc, which could be blocked by rimonabant pretreatment. This is consistent with the following putative circuitry: 2-AG acting at CB_1R on glutamatergic terminals reduces glutamate release onto GABAergic medium spiny neurons projecting to the VTA, resulting in reduced GABAergic tone which disinhibits dopaminergic neurons in the VTA, leading to increased dopamine release in the NAc (Caille et al., 2007). However, additional complexity is indicated by the presence of cholinergic interneurons in the NAc. Stimulation of these neurons directly increases DA release in coordination with PFC glutamatergic afferents without involving the VTA, and this effect is inhibited by CB_1R present on PFC terminals (Mateo et al., 2017), as discussed before. Further work is required to understand what

controls the balance between these opposing, CB_1R-mediated effects on accumbal dopamine release.

Interactions between the ECS and alcohol also occur at additional structures, such as the dorsolateral striatum (DLS) (Adermark et al., 2011b), where such interactions may influence reward-guided striatal learning (Gerdeman et al., 2002) and habit formation (Gremel et al., 2016; Hilario et al., 2007), known reinforcers of addictive behavior. Indeed, addictive behaviors, including AUD, are characterized by a degradation of the prefrontal executive control over behavior, which is shifted to the DLS resulting in the reinforcement of compulsive drug seeking behavior (Kalivas and Volkow, 2005), and a similar shift also applies to cannabis addiction (Gremel et al., 2016; Mason et al., 2012). Recent evidence indicates that chronic alcohol exposure may induce such a shift by selectively reducing the excitability of orbital frontal cortex (OFC) excitatory output to the direct output pathway in the dorsal medial striatum (DMS), which normally supports goal-directed behavior (Renteria et al., 2018).

Endocannabinoids acting via CB_1R have been implicated in the striatal drive of compulsive alcohol seeking behavior by several lines of evidence, including morphological, electrophysiological and behavioral changes in response to intermittently exposing mice to ethanol vapor (DePoy et al., 2013). Such exposure to ethanol resulted in the expansion of the dendritic arbor of DLS neurons and downregulation of DLS CB_1R signaling including a loss of CB_1R-dependent LTD. These changes were associated with a facilitation of DLS-dependent learning, as tested in a variety of behavioral paradigms. These findings indicate that the prolonged down-regulation of DLS CB_1R signaling in response to chronic alcohol exposure and the resulting DLS neuronal remodeling mediate the shift from prefrontocortical to dorsal striatal learning and the progression of alcoholism (DePoy et al., 2013). Chronic exposure to THC similarly leads to loss of endocannabinoid-mediated LTD in the DLS associated with increased habit learning, which could be rescued by blockade of small conductance, calcium activated potassium (SK) channels (Nazzaro et al., 2012). It would be interesting to test whether SK channel inhibition can similarly rescue normal, goal-directed behavior in mice chronically exposed to alcohol. A very recent study extends these findings by showing that chronic exposure of adolescent mice to alcohol causes a loss of endocannabinoid-mediated LTD in the dentate gyrus molecular layer (DGML) of the hippocampus that is coupled to impaired recognition memory. These effects last into adulthood and are associated with loss of CB_1R in the DGML, reduced CB_1R-induced G protein activation and increased expression of MAGL. Furthermore, these long-term deficits can be rescued by MAGL inhibitors (Penasco et al., 2020).

Whereas the effects of rimonabant and related CB_1R inverse agonists suggest endocannabinoid involvement, they do not prove it, as inverse agonists may produce their effects in the absence of receptor occupancy by endogenous ligands. On the other hand, a biological response to a CB_1R neutral antagonist in the absence of prior treatment with an agonist strongly indicates preexisting tonic activation by an endogenous agonist. Indeed, the CB_1R neutral antagonist AM4113 decreased binge-like alcohol drinking in male C57Bl6/J mice using a modified two-bottle drinking in the dark paradigm and attenuated alcohol-induced dopamine release in the NAc, as measured by *in vivo* microdialysis (Balla

et al., 2018). This supports the role of endocannabinoids in promoting both alcohol drinking and the release of dopamine in the NAc, as indicated by the inhibition of both by a CB_1R neutral antagonist.

Well before the above study, strong evidence for enhanced endocannabinoid/CB_1R tone maintaining high alcohol preference has been provided by multiple reports documenting reduced alcohol drinking and preference in CB_1R deficient mice. The first two studies also demonstrated age-dependence of the role of endocannabinoids in driving drinking behavior and its association with reduced G protein coupling of CB_1R in the limbic forebrain (Wang et al., 2003) and the loss of alcohol-induced dopamine release in the NAc in mice with global deletion of *Cnr1* (Hungund et al., 2003). Subsequent studies confirmed and extended these findings. CB_1R deficient mice on a mixed C57Bl/6 × 129/Ola F2 genetic background consumed less alcohol and sucrose and had greatly reduced NPY-induced food intake than their wild-type littermates, which phenocopied the effects of rimonabant (Poncelet et al., 2003). In another study using mice on a C57Bl6/J genetic background, deletion of *Cnr1* resulted in a complete loss of withdrawal symptoms and an absence of foot-shock stress-induced reinstatement of alcohol drinking (Racz et al., 2003). The inverse relationship between sensitivity to the acute intoxicating effects of alcohol and predisposition for risky drinking in human alcoholics (Schuckit et al., 2019) has been replicated in CB_1R-deficient mice whose reduced ethanol preference was associated with increased sensitivity to the sedative/hypnotic effects of alcohol (Naassila et al., 2004). CB_1R-deficient mice also display reduced conditioned place preference for alcohol (Houchi et al., 2005; Thanos et al., 2005) and increased levels of striatal D2 receptors (Houchi et al., 2005).

THERAPEUTIC POTENTIAL OF CB_1R BLOCKADE IN ALCOHOLISM

The evidence summarized above strongly implicates endocannabinoids acting via CB_1R in mediating the motivational effects of alcohol. It also documents the efficacy of CB_1R blockade/inverse agonism in attenuating alcohol preference and intake in rodent models, which had triggered two clinical studies. The first one was a multi-center, proof-of-concept, double-blind, placebo-controlled study evaluating rimonabant's efficacy in preventing relapse drinking in 260 recently detoxified alcohol-dependent patients (Soyka et al., 2008). Rimonabant at a dose of 2 × 10 mg/day for 3 months failed to produce statistically significant effects, although there was a tendency for reduced relapse rate (47.7% in the placebo group vs. 41.5% in the rimonabant group), and this trend was more marked in patients who relapsed to heavy drinking (from 35.6% to 25.7%). There was also a statistically non-significant trend toward the beneficial effects of rimonabant on secondary outcomes including abstinence duration and percentage of drinking days (Soyka et al., 2008). The second study was a double-blind, placebo-controlled trial involving 49 participants, assessing the efficacy of 20 mg/day rimonabant for two weeks in reducing drinking by non-treatment seeking heavy drinkers. Participants reported their daily alcohol intake during the 2 two weeks of call-in, followed by an 'inpatient' self-administration paradigm during which the participants had the option of consuming a total of 8 alcohol drinks. Again, there was no significant effect of rimonabant versus placebo, although there was a tendency for reduced number of drinks consumed in the inpatient component of the

study by the rimonabant group (3.3. drinks) compared to the placebo group (4.5 drinks) (George et al., 2010).

A major limitation of both studies was the use of a dose of rimonabant (20 mg/day or ~0.3 mg/kg/day) which only produces ~30% CB_1R occupancy (Huestis et al., 2001). This dosing regimen was adopted to minimize neuropsychiatric side effects that occur more frequently at higher doses. It is likely that significant reduction in alcohol drinking only occurs at complete or near-complete blockade of CB_1R, by analogy to the effects of naltrexone, a μ opioid receptor antagonist. Both compounds are believed to inhibit alcohol-seeking behavior by blocking the activity of the mesolimbic dopaminergic reward pathway. Used in the same laboratory self-administration paradigm as used by George et al., naltrexone significantly inhibited alcohol drinking at doses of 50–150 mg (O'Malley et al., 2002), which produce 100% occupancy of μ opioid receptors (Weerts et al., 2008). This suggests that the therapeutic potential of CB_1R blockade could be resurrected if one could minimize the chance of centrally mediated neuropsychiatric side effects that would allow the use of a dose producing full receptor occupancy.

One such approach may be the use of CB_1R neutral antagonists, such as AM4113, which has been found to lack some of the behavioral effects attributed to blockade of CB_1R in the CNS, such as nausea (Sink et al., 2008) or an enhanced retention of contextual fear conditioning (Sink et al., 2010a), and was less anxiogenic than the CB_1R inverse agonist AM251 (Sink et al., 2010b). However, AM4113 also induced disruptive scratching and grooming behavior in mice similar to rimonabant (Jarbe et al., 2008), a behavior considered to be a sensitive predictor of neuropsychiatric side effects in humans (Tallett et al., 2007). Further work is needed to explore the therapeutic potential and CNS safety of CB_1R neutral antagonists.

Another approach to avoid the neuropsychiatric side effects associated with CB_1R blockade in the CNS is to use second generation, peripherally restricted antagonists. The behavioral and metabolic profile of two such compounds, the neutral CB_1R antagonist AM6545 (Tam et al., 2010) and the CB_1R inverse agonist JD5037 (Tam et al., 2012) have been characterized in detail in a mouse model of diet-induced obesity. At doses causing maximal inhibition of weight gain and reversal of the metabolic consequences of obesity, neither compound elicited anxiety-like behavior in the elevated plus maze, nor induced hyperambulatory activity associated with excessive grooming and scratching or blocked cannabinoid-induced catalepsy, effects linked to CB_1R in the brain and readily induced by brain-penetrant CB_1R antagonists such as rimonabant. Unlike rimonabant, they also failed to displace a CB_1R PET ligand from binding sites in the brain.

Using such a therapeutic approach may appear counterintuitive given the undisputed role of CNS structures in the control of alcohol seeking behavior, Surprisingly, JD5037 was equally efficacious with its brain-penetrant parent compound ibipinabant in reducing food intake in mice with diet-induced obesity, another centrally regulated behavior (Tam et al., 2012). The mechanism underlying this unexpected finding turned out to involve rapid reversal of the extreme hyperleptinemia of these animals by peripheral CB_1R blockade through the reduction of leptin gene expression and secretion by blocking CB_1R in adipocytes and a

simultaneous increase in the renal clearance of leptin by blocking CB_1R in proximal tubular cells (Tam et al., 2012). In agreement with the role of hyperleptinemia in maintaining leptin resistance in obesity (Knight et al., 2010), its rapid reversal by peripheral CB_1R blockade resulted in restoration of leptin sensitivity, so the observed hypophagia and associated weight loss could be attributed to endogenous leptin. Accordingly, CB_1R blockade failed to reduce food intake and body weight in leptin deficient *ob*/*ob* mice, and in diet-induced obese mice these effects could be inhibited by a pegylated leptin receptor antagonist (Tam et al., 2012).

A recent study explored whether a similar periphery-to-brain axis may be involved in regulating alcohol seeking behavior, using the peripheral CB_1R inverse agonist JD5037 in a two bottle/free choice as well as a limited access, drinking in the dark paradigm, and male mice on a C57Bl6/J background (Godlewski et al., 2019). JD5037 treatment significantly reduced ethanol preference and intake in wild-type mice but not in mice deficient in CB_1R, ghrelin, or the ghrelin receptor $GHSR_{1A}$, indicating that the stomach-derived hormone ghrelin may be the link between peripheral CB_1R and the CNS structures controlling alcohol drinking behavior. JD5037 treatment of alcohol-drinking mice inhibited the formation of biologically active octanoyl-ghrelin but not its precursor desacyl-ghrelin, suggesting that CB_1R modulate ghrelin acylation, catalyzed by the enzyme ghrelin O-acyl transferase or GOAT. This was supported by the reduced alcohol preference which was unaffected by JD5037 treatment in mice deficient in *Mboat4*, the gene encoding GOAT. Ghrelin-producing MGN3–1 mouse stomach ghrelinoma cells express CB_1R and generate high levels of endocannabinoids (Godlewski et al., 2019), similar to primary ghrelin-producing cells of the stomach mucosa (Engelstoft et al., 2013). In MGN3–1 cells incubated with deuterated palmitic acid, JD5037 reduced the level of the substrate octanoyl-carnitine generated from palmitoyl-carnitine by increasing the rate of fatty acid β-oxidation, which reduced the amount of octanoyl-ghrelin generated by these cells. This indicated that blockade of CB_1R in ghrelin-producing cells reduces ghrelin-acylation by reducing the availability of its substrate octanoic acid. Finally, blocking vagal afferents, which express $GHSR_{1A}$, abrogated the ability of either CB_1R or $GHSR_{1A}$ blockade to reduce alcohol drinking. This suggests that octanoyl-ghrelin released by ghrelin-producing cells activates $GHSR_{1A}$ on neighboring vagal afferent terminals to generate a neural signal to brain structures involved in the control of alcohol drinking behavior (Godlewski et al., 2019). This mechanism is schematically illustrated in Figure 1.

Interestingly, vagal afferent nerve firing is decreased by signals that increase alcohol intake, such as ghrelin (Date et al., 2002), whereas it is increased by blockade of peripheral ghrelin receptors (Kong et al., 2016) or by cholecystokinin (Date et al., 2002; Schwartz et al., 1997), which also reduces alcohol intake (Geary et al., 2004), The apparent reciprocal relationship between vagal afferent nerve firing and alcohol preference is compatible with the increased incidence of alcoholism following Roux-en-Y gastric bypass surgery, which disrupts gastric innervation (Hajnal et al., 2012), and results in loss of ghrelin regulation of the firing of VTA dopaminergic neurons (Sirohi et al., 2017).

As discussed above, the use of a suboptimal dose of rimonabant in previous clinical trials in order to minimize the risk of CNS-mediated adverse effects was a possible reason for its

failure to elicit statistically significant therapeutic effects in AUD. As such a dose limitation does not apply to peripherally restricted CB_1R antagonists, future clinical trials to test their therapeutic potential in AUD are warranted.

POSSIBLE INVOLVEMENT OF CB_2R IN ALCOHOL DRINKING BEHAVIOR

Although low level expression of CB_2R mRNA in the brain has been detected in multiple laboratories (Onaivi et al., 2012; Van Sickle et al., 2005), direct evidence for the presence of CB_2R protein in neuronal cells is lacking (Lopez et al., 2018). Nevertheless, some electrophysiological evidence is compatible with the presence of functional CB_2R in CNS neurons (Atwood and Mackie, 2010; Stempel et al., 2016). There is limited and conflicting information about the possible involvement of CB_2R in the control of alcohol seeking behavior. CB_2R knockout mice displayed increased ethanol-induced conditioned place preference (CPP), increased voluntary ethanol intake, increased motivation to drink ethanol and increased acquisition of ethanol self-administration, relative to wild-type controls (Ortega-Alvaro et al., 2015). The phenotype of the knockouts could be replicated in wild-type mice by the similar increase in alcohol-induced CPP and consumption elicited by the CB_2R antagonist AM630 and the opposite effects of the agonist JWH633 (Navarrete et al., 2018). The increased ethanol CPP in CB_2R KO mice was confirmed by others, although in this study the effects of the genetic knockout could not be replicated by CB_2R antagonist treatment (Powers et al., 2015). Further complicating the picture, another group recently reported that both AM630 and JWH633 decrease alcohol reward as measured by alcohol-induced CPP (Martin-Sanchez et al., 2019). Data obtained in genetic knockouts also fall short of delivering conclusive evidence for the role of CB_2R in alcohol seeking behavior. In contrast to the increased alcohol preference in mice with global knockout of CB_2R, conditional knockout of the *Cnr2* gene in midbrain dopaminergic neurons resulted in decreased preference for alcohol and decreased alcohol-induced CPP. In the same study, treatment of wild-type littermate mice with the CB_2R agonist JWH-133 similarly reduced alcohol-induced CPP, which is difficult to interpret (Liu et al., 2017). Further work is clearly needed to convincingly implicate CB_2R in the control of alcohol reward and alcohol seeking behavior.

ENDOCANNABINOIDS AND ALCOHOLIC LIVER DISEASE

Contrary to earlier notions, hepatocytes were found to express functional CB_1R, activation of which promotes hepatic insulin resistance, stimulates *de novo* lipogenesis by inducing the lipogenic transcription factor SREBP1c and its targets acetyl co-A carboxylase and fatty and synthase, and reduces fatty acid oxidation and (Osei-Hyiaman et al., 2005; Osei-Hyiaman et al., 2008). Indeed, rimonabant was effective not only in reducing body weight in overweight people with the metabolic syndrome but, first among anti-obesity agents, simultaneously improved all of the major metabolic complications, including non-alcoholic fatty liver disease (NAFLD), dyslipidemia and insulin resistance (Despres et al., 2005; Gary-Bobo et al., 2007; Pi-Sunyer et al., 2006; Van Gaal et al., 2005). This supports the notion that increased activity of the endocannabinoid/CB_1R system is a pathogenic feature of the metabolic syndrome.

Chronic alcoholism can lead to alcoholic fatty liver disease (AFLD), the underlying molecular mechanisms of which are similar to those involved in NAFLD, including increased *de novo* lipogenesis (Lieber et al., 1966; You et al., 2002), decreased fatty acid oxidation (You et al., 2004) and insulin resistance (Carr et al., 2013). It is therefore not surprising that endocannabinoids acting at CB_1R were found to be major drivers of AFLD (Jeong et al., 2008). This conclusion was based on the findings that mice deficient in CB_1R globally or in hepatocytes only are resistant to ethanol-induced steatosis and increased lipogenic gene expression and have increased activity of hepatic carnitine palmitoyltransferase-1, the rate limiting enzyme in fatty acid β-oxidation. Furthermore, ethanol feeding increased hepatic *Cnr1* expression and increased the levels of 2-AG (but not anandamide) along with increased gene expression of its biosynthetic enzyme DAGLβ selectively in hepatic stellate cells (HSC). In addition, co-culture of wild-type but not $Cnr1^{-/-}$ hepatocytes with HSC from alcohol drinking mice resulted in upregulation of *Cnr1* and lipogenic gene expression in the hepatocytes (Jeong et al., 2008). This supported the hypothesis that paracrine activation CB_1R on hepatocytes by HSC-derived 2-AG mediates alcoholic steatosis.

The above findings left open the question of the mechanism by which alcohol drinking increases 2-AG production by HSC. This question has just been answered by recent findings that chronic ethanol intake induces hepatic cysteine deficiency and subsequent glutathione depletion, triggering a compensatory increase in the gene and protein expression of the hepatic cystine/glutamate antiporter xCT which results in increased extracellular levels of glutamate. Alcohol intake also induces the expression of the metabotropic glutamate receptor-5 (mGluR5) selectively in HSC, stimulation of which by the released glutamate induces the production and release of 2-AG by HSC to activate CB_1R and triggering *de novo* lipogenesis in neighboring hepatocytes (Choi et al., 2019). This bidirectional metabolic synapse (schematically illustrated in Figure 2), which is reminiscent of the bidirectional communication in the brain via anterograde and retrograde synaptic transmission, reveals novel targets for the treatment of AFLD. Indeed, genetic deletion or pharmacologic inhibition of either xCT or mGluR5 mitigated alcohol-induced steatosis (Choi et al., 2019).

Interestingly, both these proteins have been implicated in the control of alcohol drinking behavior. Certain β-lactam antibiotics were found to reduce voluntary alcohol intake in P rats and in parallel to increase astrocytic expression of xCT in the PFC and NAc (Alasmari et al., 2015), suggesting that inhibition of xCT activity could increase alcohol preference and drinking, an undesirable effect opposite to the beneficial reduction in alcoholic steatosis. In contrast, mGluR5 inhibition reduces alcohol-seeking behavior both in mice and alcoholic patients (Bird et al., 2008; Holmes et al., 2013), similar to its beneficial inhibitory effect on hepatic steatosis, as discussed above. Thus, engaging the same pharmacological target (mGluR5) can mitigate alcoholism as well as alcoholic liver disease. The same principle may apply to peripherally restricted CB_1R antagonists, which may not only be effective in curbing the drive to drink alcohol via a stomach/brain axis (Godlewski et al., 2019), but have also been shown to mitigate alcohol-induced steatosis (Amato et al., 2018). Furthermore, the mechanism identified in mice may also operate in human alcoholism, as suggested by the finding of increased plasma glutamate levels in patients with AFLD and alcoholic steatohepatitis compared to healthy controls or patients with cirrhosis, and changes in gene

expression identified in the livers of alcohol-fed compared to control mice were also evident in liver tissue from patients with AFLD vs. controls without steatosis (Choi et al., 2019).

Cytochrome P450 2E1 (CYP2E1) is a key enzyme involved in alcohol-induced generation of reactive oxygen species (ROS) which cause oxidative stress-related tissue damage. The pathway linking alcohol and increased expression of CYP2E1 involves CB_1R and the nuclear receptor estrogen-related receptor γ (ERRγ), as indicated by the absence of alcohol-induced CYP2E1 expression and ROS production in *Cnr1*$^{-/-}$ mice or mice with liver-specific deletion of the gene encoding EERγ (Kim et al., 2013). Alcohol-induced steatosis and ROS-mediated tissue damage can both progress to the development of liver fibrosis, so it is not surprising that the ECS has been implicated in hepatic fibrogenesis (Siegmund, 2010; Teixeira-Clerc et al., 2010; Teixeira-Clerc et al., 2006), including alcoholic liver fibrosis (Patsenker et al., 2011; Trebicka et al., 2011), with CB_1R and CB_2R being pro- and antifibrogenic, respectively (Mallat et al., 2013).

In contrast to the role of CB_1R in promoting ALD, activation of CB_2R may protect against alcoholic steatohepatitis. The CB_2R agonist JWH-133 mitigated alcohol-induced hepatic inflammation and steatosis by suppressing pro-inflammatory M1 polarization and promoting the M2 phenotype of Kupffer cells (Louvet et al., 2011). The CB_2R-mediated anti-inflammatory effects in Kupffer cells were subsequently found to involve activation of the autophagy pathway (Denaes et al., 2016). B-Caryophyllene (BCP) is a plant-derived substance with CB_2R agonist and anti-inflammatory properties (Gertsch et al., 2008). When chronic plus binge alcohol-fed mice were chronically treated with BCP, the hepatic steatosis and inflammatory changes such as neutrophil infiltration and M1 phenotypic switch of Kupffer cells were significantly ameliorated, and these effect were more prominent in wild-type than in $CB_2R^{-/-}$ mice, indicating a protective effect mediated by CB_2R activation (Varga et al., 2018). Together, these findings point to the therapeutic potential of CB_2R agonists in ALD.

ENDOCANNABINOIDS, CB_1R AND ALCOHOL BINGE-INDUCED CARDIOVASCULAR DYSFUNCTION

Endocannabinoids have robust effects on the cardiovascular system, predominantly via activation of CB_1R. CB_1R are present in both the rodent (Batkai et al., 2007) and human heart (Valenta et al., 2018) where its activation reduces cardiac contractility (Batkai et al., 2007), whereas activation of CB_1R in the vascular endothelium leads to long-lasting, profound vasodilation and hypotension (Batkai et al., 2001; Lake et al., 1997). In a recent study in mice, a single dose of 5 g/kg ethanol delivered by gavage elicited a profound decrease in cardiac contractility, detected 3 hours later by intraventricular pressure-volume catheterization and echocardiography and paralleled by an increase in myocardial anandamide (but not 2-AG) levels. This was associated with hypotension and a redistribution of blood flow from the kidneys and mesenteric bed to skeletal muscle, the latter probably resulting from a reflex increase in sympathetic tone. All these changes were reversed or attenuated by a bolus dose of rimonabant, indicating CB_1R activation as the underlying mechanism (Paloczi et al., 2019).

SUMMARY

Endocannabinods are ubiquitous lipid mediators involved in the control of a broad range of biological functions both in the CNS and in peripheral tissues. In the brain, endocannabinoid signaling via CB_1R in the mesolimbic dopaminergic pathway mediate drug including alcohol reward, whereas CB_1R-signaling in the dorsolateral striatum has been implicated in compulsive habitual alcohol-seeking behavior. In the liver, CB_1R in hepatocytes play a key role in alcoholic fatty liver disease and CB_1R in cardiomyocytes have been linked to binge alcohol-induced acute cardiovascular disturbance. Blockade of CB_1R mitigates all these effects of alcohol drinking but its therapeutic exploitation has been thwarted by CNS-mediated side effects. Recently developed, peripherally restricted CB_1R antagonists have been shown to retain all the beneficial effects of global CB_1R blockade, including the reduction of voluntary alcohol intake via a stomach/brain signaling axis, without its neuropsychiatric liability. Such compounds therefore have therapeutic potential in the management of both addictive drinking as well as the organ toxicity associated with chronic alcoholism.

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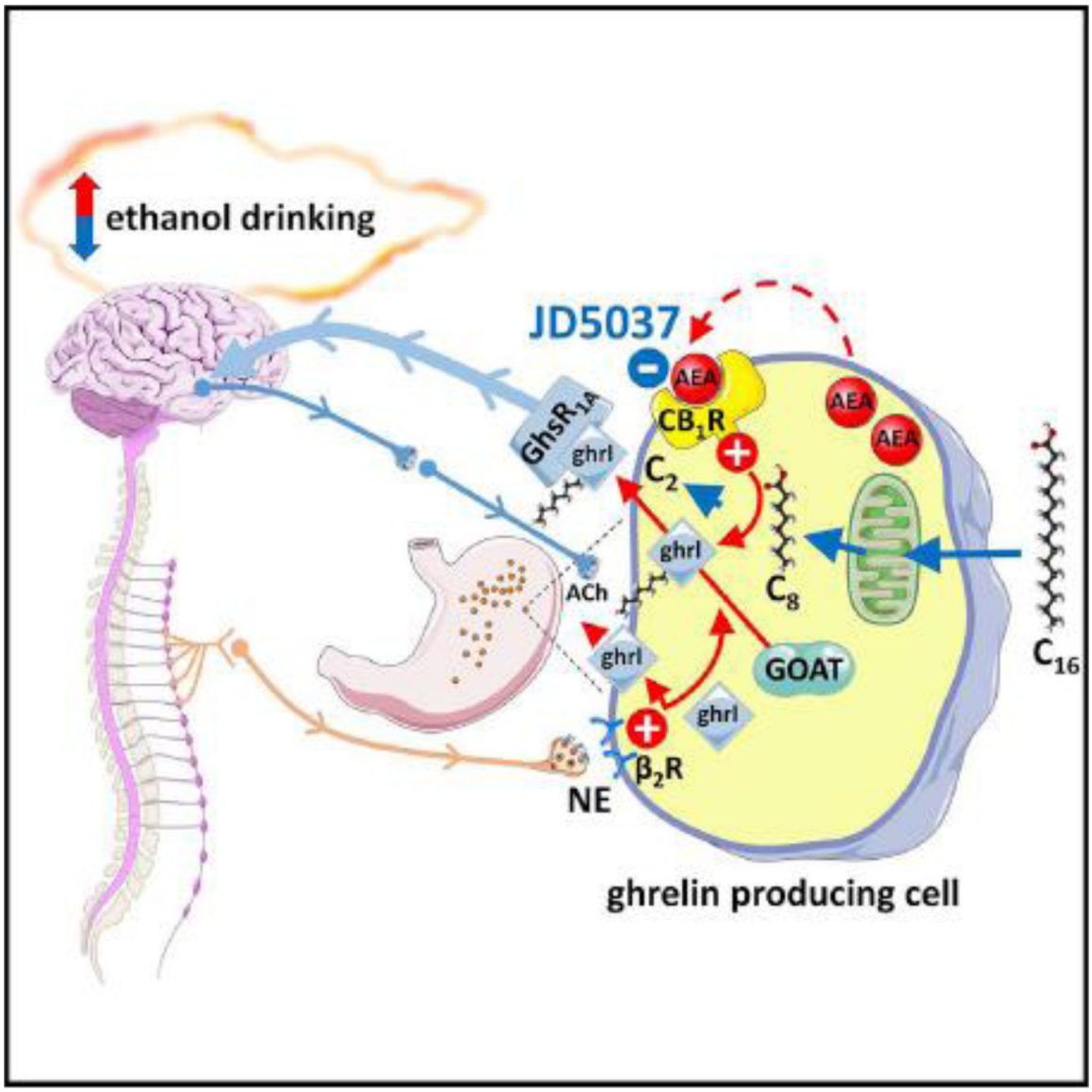

Figure 1. Endocannabinoids modulate alcohol drinking behavior via a stomach/brain axis.

In ghrelin-producing stomach cells, the enzyme ghrelin O-acyl transferase (GOAT) catalyzes the formation of biologically active octanoyl-ghrelin. The source of the octanoic acid (C8) substrate is circulating long-chain fatty acids such as palmitate (C16) which are taken up by the cells and subjected to acyl chain shortening via β-oxidation. Blockade of CB_1R on ghrelin-producing stomach cells accelerates the rate of fatty acid β-oxidation, thus reducing the availability of C8 for ghrelin acylation and, consequently, the cellular level of octanoyl-ghrelin without affecting its precursor deasacyl ghrelin. Octanoyl-ghrelin released by the cells activates ghrelin receptors (GhsR1A) located on vagal afferent terminals that signal to the brain to reduce drinking. In contrast to the selective modulation of octanoyl-ghrelin levels by CB_1R, norepinephrine (NE) released from sympathetic nerves and activating β_2-adrenergic receptors (β_2R) stimulates the release of both desacyl- and

octanoyl-ghrelin, which also promotes alcohol drinking that can be antagonized by the βR-blocker propranolol.
Reproduced from Godlewski et al., Cell Metabolism 29:1320–33, 2019.

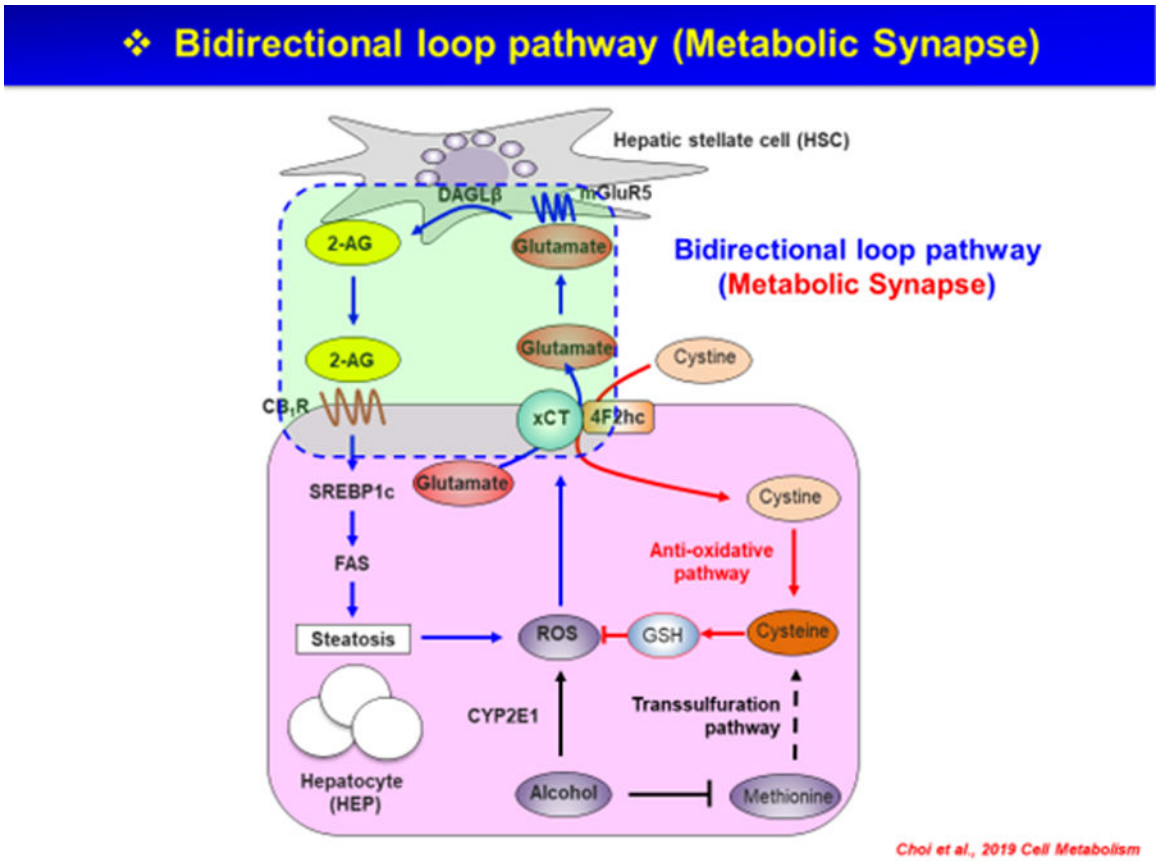

Figure 2. A 'bidirectional metabolic synapse' between hepatocytes and hepatic stellate cells (HSC) mediates endocannabinoid-induced *de novo* lipogenesis.

Chronic alcohol consumption induces CYP2E1-mediated production of reactive oxygen species (ROS) by hepatocytes, which is compensated by glutathion (GSH) generation through the uptake of cystine, mediated by the cystine-glutamate antiporter xCT. The parallel release of glutamate stimulates metabolic glutamate receptor-5 (mGluR5) on hepatic stellate cells (HSC) to produce 2-arachidonoyl glycerol (2-AG). 2-AG then activates CB_1R on adjacent hepatocytes to induce *de novo* lipogenesis by inducing the lipogenic transcription factor SREBP-1c and the lipogenic enzyme fatty acid synthase (FAS). Reproduced from Choi et al., Cell Metabolism, 30:877–889, 2019.