Opioid and Cannabinoid Systems as Therapeutic Targets for the Treatment of Alcohol Dependence: From Animal Models to Clinical Practice

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Abstract: The development of alcohol dependence is the result of a combination of various factors. Psychosocial and psychiatric conditions, together with functional alterations of the brain or genetic traits, contribute to the development of problems related to alcohol use or alcohol dependence. Clinical studies using neuroimaging techniques (PET, fMRI) and preclinical studies using different animal models of problems related to ethanol consumption have improved our knowledge of the neurochemical mechanisms involved in alcohol dependence. These studies have served to identify peptides or receptors modified by ethanol consumption, which are functionally altered in strains of rats or mice highly vulnerable to ethanol consumption. Such peptides or receptors may be interesting targets for the treatment of alcoholism.

Among the different targets studied in recent years, the opioid and cannabinoid systems meet a number of conditions for eligibility as candidates for the treatment of alcohol dependence. The μ-opioid receptor and cannabinoid CB1 receptor, in particular, are affected by ethanol consumption. In clinical studies, genetic polymorphisms of the μ-opioid and CB1 receptors have been associated with increased vulnerability to alcohol consumption. Similarly, functional alterations in μ-opioid and cannabinoid receptors have been identified in specific strains of rats or mice with high preference to ethanol consumption. Furthermore, several studies have shown that the manipulation of these receptors using agonists or antagonists may increase or decrease ethanol consumption, which confirms the validity of these receptors as targets for the treatment of alcohol dependence. In this review, we analyzed the genetic traits and psychiatric and/or psychosocial conditions that affect vulnerability to and the pharmacologic treatment of alcohol dependence, with special emphasis on the role of opioid and cannabinoid receptors. The use of animal models as important tools for identifying neurochemical mechanisms relevant to understanding and treating alcohol use problems was evaluated.

1. INTRODUCTION

Excessive alcohol consumption is a complex multifactorial problem that leads to loss of neurochemical homeostatic control in the brain. A number of psychosocial conditions, such as high impulsivity, low self-esteem, sensation-seeking behavior, phobias, and affective disorders, may favor the development of problems related to alcohol use or facilitate the progression to alcohol dependence. Considerable evidence has emerged that suggests that the reinforcing properties of drugs of abuse are due to activation of a common reward pathway involving brain dopamine (DA) neurotransmission [1]. Drugs of abuse, such as alcohol, stimulate DA neurons in the ventral tegmental area (VTA) [2, 3], increasing DA release in the nucleus accumbens (NAcc) [4-7]. However, the acquisition and maintenance of alcohol drinking behavior depend not only on mesolimbic DA neurons, but also on neuronal systems, such as the opioid, cannabinoid, GABA, glutamate, nAChR/glycine, DA/5-HT, and CRF/NPY neurons [8], and involve other brain regions, including the amygdala (Amy), cortex, and hippocampus [4, 9, 10].

The most important system closely involved in the development of alcohol dependence is the opioidergic system. Dopaminergic neurons, whose cell bodies are located in the VTA and axonal terminals in the NAcc, are under tonic GABAergic inhibition [7]. GABA interneurons may be disinhibited by activation of μ-opioid receptors [11]. Ethanol stimulates the release of the opioid peptides β-endorphin and enkephalin, and this may lead to increased DA release in the NAcc [12]. In contrast, stimulation of the κ-opioid receptor by dynorphin peptides decreases DA release and may produce aversive states that probably prevent reinforcement [6, 13].

In recent years, evidence has accrued suggesting that the endogenous cannabinoid system has an important role in the regulation of ethanol intake [14-16]. Moreover, several studies found functional alterations in the cannabinoid system, in areas of the reward pathway, produced by acute and chronic ethanol intake [17-21]. Interestingly, the

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administration of opioid agonists or antagonists by the infusion of exogenous cannabinoid ligands produces modifications in several elements of the cannabinoid system and opioid system [22-25], suggesting an interaction between these two systems. In conclusion, a better understanding of the complex interactions between alcohol and the endogenous opioid and cannabinoid systems may help to identify new ways to increase the effectiveness of therapies for alcohol dependence.

Therefore, in the present review we will summarize the new advances integrating the role of these two endogenous neurochemical systems, the opioid and cannabinoid systems, in the vulnerability to and development of alcohol dependence. We will emphasize the genetic traits and psychiatric or psychosocial conditions that underlie alcohol dependence (Fig. **1**), as well as pharmacologic treatment based on emerging translational research.

2. VULNERABILITY TO ALCOHOL USE DISORDERS

Psychosocial and psychiatric conditions, such as stress and anxiety, together with functional alterations of the brain and certain genetic traits (*i.e.*, the genetic background) contribute to the development of problems related to alcohol use or alcohol dependence in humans and in animal models. Theoretical and empirical evidence indicates that anxiety and stress-related disorders increase vulnerability to alcohol use problems and the development of dependence [26]. The general belief is that alcohol reduces stress [27] and that alcohol intake increases during stressful situations [28]. Experimental research shows that stress activates the hypothalamic-pituitary-adrenal (HPA) axis, which allows glucocorticoid-mediated sensitization of the reward pathway [29, 30]. It is plausible that variations in glucocorticoid levels between individuals with different HPA axis functional activity may be responsible for the distinct vulnerability that occurs in alcohol dependence [31]. Furthermore, the neurotransmitter systems that modulate both mesolimbic DA pathways and corticotropin-releasing hormone secretion may contribute to genetic vulnerability to alcoholism and/or HPA dysfunction. Stress also activates endogenous opioids [32], which may subsequently facilitate DA release and increase the vulnerability to relapse of alcohol dependence [33]. However, animal studies dedicated to analyzing the effect of stress and glucocorticoids on the initiation of ethanol drinking have been less extensive than for other psychostimulants, such as cocaine. In fact, studies in non-ethanol dependent rodents have elicited less solid results than ethanol deprivation models testing the effects of the same types of stress on ethanol intake and stress-induced increases in ethanol intake [31]. Despite these limitations, it is likely that the influence of stress on voluntary ethanol intake by rodents depends on the type of stress applied and the genetic background of animals [34, 35]. In addition, vulnerability to increased ethanol consumption is determined more by individual differences in the sensitivity of the opioid system to ethanol than by differences in the baseline levels of endogenous opioid activity [36]. On the other hand, little is known about the vulnerability of the endocannabinoid system to stress and ethanol consumption or the neurochemical implications of decreasing ethanol intake (see [37] for review). Previous findings by our group suggest that the cannabinoid CB1 receptor is related to increased vulnerability to voluntary ethanol consumption in ethanol-preferring fawn-hooded versus ethanol non-preferring Wistar rats [21]. In this study, a decrease in cannabinoid CB1 receptor function and gene expression was observed in brain areas related to reward processes, which highlights the important role of the CB1 receptor in ethanol vulnerability and related disorders. Thus, it has been demonstrated that foot-shock stress had no effect on ethanol preference in cannabinoid CB1 receptor knockout mice, although it induced a dramatic increase in cannabinoid CB1 receptor in wild-type animals [38]. These results suggest a crucial role

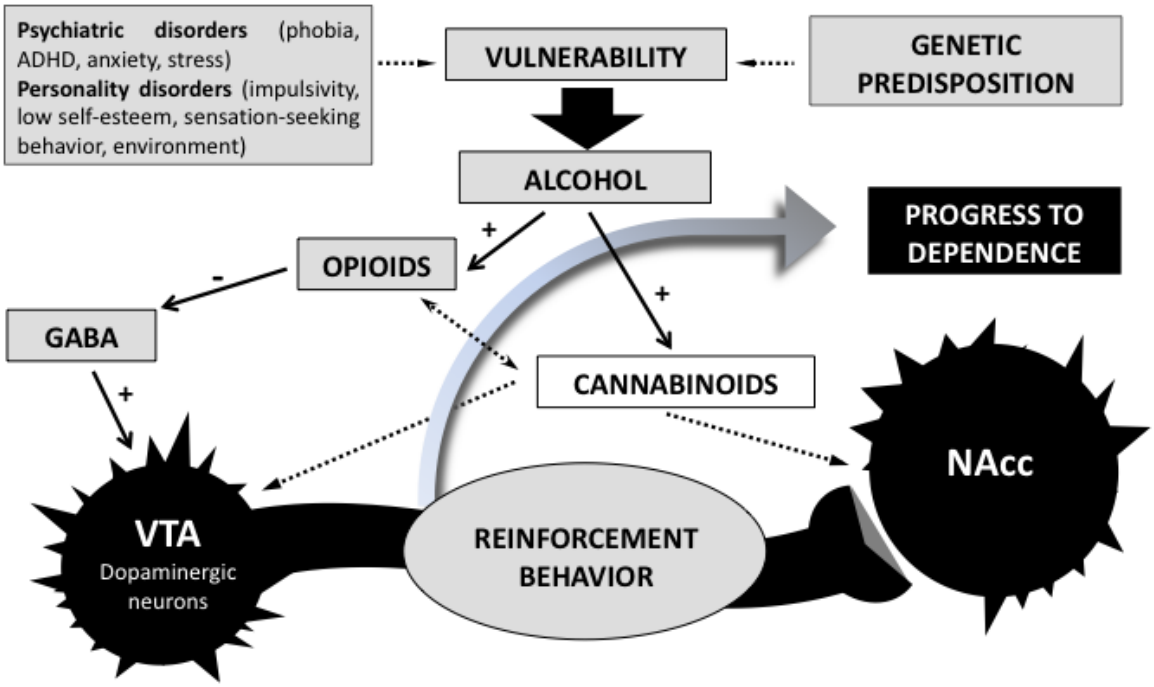

Fig. (1). Role of psychiatric and genetic traits in vulnerability to alcohol consumption and dependence.

of the CB1 receptor in the regulation of ethanol dependence and in stress-induced increased ethanol preference.

Anxiety is another condition implicated in vulnerability to alcoholism and alcoholism co-morbidity [39]. Spanagel *et al.* claim that the baseline level of anxiety plays an important role in vulnerability to ethanol drinking in rats [40] and in non-human primate models [41, 42]. Other preclinical studies using different types of animal models with problems related to ethanol intake have improved our knowledge of neurochemical mechanisms involving opioid and cannabinoid systems in alcohol dependence [37]. These studies have served to identify opioid and cannabinoid ligands, or receptors, that are modified by ethanol intake, are functionally altered in different strains of rats or mice highly vulnerable to ethanol consumption, and, therefore, could be interesting targets for the treatment of alcoholism. Baseline proopiomelanocortin (POMC) gene expression is increased in selectively bred AA (ethanol preferring) rats [43] and in ethanol preferring C57BL/6 mice compared to ethanol non-preferring DBA/2 mice [44]. Met-enkephalin and leu-enkephalin peptide levels in the NAcc are lower in AA rats than in ANA rats [45], whereas more proenkephalin gene expression is reported in the prefrontal cortex of AA rats than ANA rats [43]. Fawn-hooded rats showed a strong preference for ethanol intake (10% v/v) in a two-bottle free-choice situation [21, 46] that may be related, at least in part, to decreased brain opioid function [47]. Lower μ-opioid receptor-stimulated [^{35}S]γGTP binding autoradiography in the caudate putamen (CPu) and cingulate cortex, lower proenkephalin gene expression in the CPu and NAcc, and lower POMC gene expression in the arcuate nucleus have been found in fawn-hooded compared to Wistar rats [21]. The findings from human studies have largely paralleled preclinical findings [48].

Genetic engineering also has boosted the development of animal models for the in-depth study of alcohol-related genes [49], including elements of the opioid and cannabinoid systems. Mice lacking μ-opioid receptors did not self-administer ethanol [50], whereas δ-opioid receptor knockout mice showed more ethanol preference and ethanol consumption [51]. Moreover, cannabinoid CB1 receptor knockout mice drank less ethanol than their wild-type littermates [52-56] and showed more ethanol sensitivity and more withdrawal severity [57], although a paper by Racz *et al.* has shown that ethanol withdrawal symptoms were completely absent in cannabinoid CB1 receptor-deficient mice [38]. Because of this, μ-opioid and cannabinoid CB1 receptor antagonists may be useful for reducing alcohol intake.

Studies of ethanol-related polymorphisms involving the opioid system in animal models are limited and only cover analyses of variants of the κ-opioid receptor gene in different strains of mice that could be a significant source of phenotypic variation in alcohol preference [58]. This is not the case of human genetic studies analyzing opioid system polymorphisms. Epidemiologic evidence strongly supports the idea that differences in opioid activity confer inherited vulnerability to alcoholism in human populations [59-61]. In fact, considerable progress has been made in recent years by molecular biologists in identifying genetic polymorphisms that may be responsible for functional differences in the endogenous opioid system and putative risks for alcoholism [62]. Because of the physiologic relevance of the μ-opioid receptor, much of this work has targeted this gene [63]. The main polymorphism of interest involves a common A118G nucleotide exchange in exon 1 of the μ-opioid receptor gene that causes Asn40Asp substitution in the extracellular N-terminal domain of the receptor [64-68]. Taken together, these studies suggest that the A118G single nucleotide polymorphism (SNP) alters the HPA-axis and DA function in response to pharmacologic opioid antagonism and alcohol administration.

In relation to endocannabinoid system polymorphisms and ethanol-related problems, research has focused mainly on human populations and genetic association studies. Cannabinoid CB1 receptor gene polymorphisms have been related to childhood attention deficit/hyperactivity disorder (ADHD) in patients with alcoholism. These data are consistent with the fact that the cannabinoid system modulates dopaminergic transmission, and that disruption of the dopaminergic system is a potential physiopathologic cause of ADHD [69]. More recently, SNPs of the cannabinoid CB1 receptor gene, especially rs6454674 and rs806368, were found to be associated with alcohol and illicit drug dependence in European Americans [70]. Nonetheless, the initial hypothesis that genetic variants of the cannabinoid CB1 receptor gene might be associated with susceptibility to alcohol use disorders [71, 72] has unveiled ambiguous data. Firstly, Schmidt *et al.* [73] analyzed a silent polymorphism (1359G/A; Thr453Thr) in the single coding exon of the human cannabinoid CB1 receptor gene and found that the homozygous genotype confers vulnerability to alcohol withdrawal delirium, although this outcome was not confirmed by others [74]. Secondly, Herman *et al.* [75] failed to replicate the original report [72] of an association between SNPs adjacent to an alternative cannabinoid CB1 receptor gene exon 3 transcription start site and polysubstance abuse.

Recently, a relationship has been suggested between Q63R polymorphism of the CB2 gene and alcoholism in Japanese subjects [76], as well as with depression and substance use disorders [77, 78]. Finally, genetic analysis of the principal endocannabinoid-inactivating enzyme, fatty acid amide hydrolase (FAAH), showed that a missense mutation (Pro129Thr) yields an enzyme with enhanced sensitivity to proteolysis, which may be associated with alcohol abuse and dependence [79], and with alcohol use disorder and antisocial personality disorder comorbidity [80].

3. EFFECTS OF ALCOHOL CONSUMPTION ON THE OPIOID SYSTEM

A large body of experimental evidence has established that ethanol interacts with the opioid system, inducing the release of opioid peptides [12, 81] and modifying the expression and function of opioid receptors [82, 83]. In fact, acute ethanol administration increases endorphin and enkephalin levels in discrete brain regions and the pituitary of rodents [84-88], and proenkephalin gene expression in the CPu, NAcc, Amy, and ventromedial and paraventricular hypothalamic nucleus [3, 89]. Other studies using *in situ* hybridization after chronic ethanol consumption disclose no

change in proenkephalin gene expression in the rostral striatum of rats [90]. However, other authors have found decreased preproenkephalin mRNA levels in the NAcc and olfactory tubercle in rats and increased preproenkephalin mRNA levels in central and intercalated nuclei of the Amy [91]. Furthermore, a recent study shows that acute ethanol administration also produces decreased levels of proenkephalin gene expression in the substantia nigra [89]. POMC increases after the administration of ethanol [92, 93] and an ethanol-containing diet [94, 95]. In contrast, several groups have demonstrated decreases in POMC following an inhalation chamber paradigm, liquid diet and intragastric administration [96-98]. Chronic ethanol administration studies show decreased POMC gene expression in the forebrain [99] and pituitary gland of rats [87] and β-endorphin release in cultured hypothalamic neurons [100]. In addition, marked changes in the μ-opioid receptor are found after different chronic ethanol paradigms. For instance, chronic voluntary ethanol intake (50 days) inhibits DAMGO-stimulated [^{35}S]γGTP binding in the NAcc, CPu, and lateral septum of fawn-hooded ethanol-preferring rats [101]. In contrast, μ-opioid-stimulated [^{35}S]γGTP binding is lower in the prefrontal cortex of brains from ethanol self-administering Long Evans rats, although voluntary ethanol intake showed no effect on μ-opioid function in the cingulate cortex, CPu, NAcc, Amy, hypothalamus, and locus coeruleus compared with sucrose self-administering rats [102]. A model of prolonged ethanol consumption (30 days) increased μ-opioid receptor binding in the CPu of Sardinian rats [82], but down-regulated these receptors in the NAcc and CPu of Wistar rats [83]. These discrepancies may be due to methodologic differences in ethanol administration, the amount of ethanol consumed, duration of treatment, and the animal species or strain selected.

4. EFFECTS OF ALCOHOL CONSUMPTION ON THE CANNABINOID SYSTEM

The endogenous cannabinoid system comprises endogenous ligands (anandamide, 2-AG, noladin ether, virodhamine, and N-arachidonoyl dopamine), cannabinoid synthesis, transport and degradation enzymes (*e.g.*, FAAH), and cannabinoid CB1 and CB2 receptors [103, 104].

Animal models and genetic deletions in the CB1 receptor have been the most relevant tools for studying the effects of pharmacologic activation or blockade of the cannabinoid system. Pharmacologic manipulation of the CB1 receptor shows that the administration of cannabinoid agonists increases ethanol intake and preference in rodents [56, 105, 106], whereas cannabinoid antagonists decrease these actions [14, 107]. Knockout mice for the cannabinoid CB1 receptor display less ethanol consumption than their wild-type littermates [56] and reduced ethanol-induced conditioned place preference, self-administration in a two-bottle choice paradigm [54, 108], and ethanol neurotoxic susceptibility in infant CB1 receptor knockout mice [109]. It is important to note that these findings are not related to enhanced ethanol metabolism [55]. In relation to ethanol withdrawal symptoms in CB1 knockout mice, it has been shown some contradictory data although using different strain background and methodology [38, 57].

Alterations of cannabinoid CB1 receptor gene expression in selected areas of the rat brain after acute, chronic and self-administering paradigms of ethanol intake may contribute to triggering the rewarding effects of ethanol consumption. In fact, bidirectional changes have been observed in ethanol self-administration tests with CB1 receptor agonists and antagonists, indicating that ethanol reinforcement might be controlled by CB1 receptors in ethanol-preferring rats, and demonstrating that endocannabinoids and their receptors mediate ethanol reinforcement [110]. Other findings supporting the involvement of the cannabinoid system in ethanol dependence include alterations in the synthesis of endocannabinoids, their precursors, and in the density and binding efficacy of CB1 receptors [111]. In this case, it is noteworthy that different routes of administration of ethanol resulted in different outcomes. Chronic and forced ethanol administration down-regulated cannabinoid receptors in mouse brain [21] and increased levels of endogenous anandamide and its precursors [112]. However, a single dose of ethanol decreased endocannabinoid levels [113-115]. Moreover, CB1 receptor levels diminished markedly in rats with acute ethanol intake only in the Amy and prefrontal cortex. These results suggest that reductions in endocannabinoid and N-acylethanolamine levels may not be caused by enhanced FAAH enzyme activity, but associated with low levels of the receptors activated by endogenous cannabinoid ligands. These results are observed specifically in the brain regions implicated in stress, emotion, and feeding, but not in motor-related areas. These observations support the notion of a general reduction in endocannabinoid signaling activity as a result of acute ethanol exposure [114].

Little information is available about the role of cannabinoid CB2 receptor in substance use disorders. Recently, it was hypothesized that stress and depression are associated with an alteration in cannabinoid CB2 receptor gene expression that may also be involved in the effects of drugs of abuse. Thus, mice that develop ethanol preference present reduced CB2 receptor gene expression whereas chronic treatment with CB2 receptor agonists (*i.e.*, JWH015) enhanced ethanol consumption only under stressful conditions [77]. These findings suggest new and promising leads for research in alcohol-related problems.

5. OPIOID PHARMACOLOGY IN ALCOHOL CONSUMPTION

The enhanced activity of the endogenous opioid system produced by alcohol consumption may be explained, at least in part, by the ethanol-induced reward response. In addition, pharmacologic manipulation of opioid receptors affects the rewarding properties of ethanol and the acquisition and maintenance of ethanol consumption, as has been observed in several experimental studies. Therefore, the blockade of central opioid receptors using selective and non-selective opioid antagonists may modulate the positive reinforcing properties of ethanol [116, 117] and be effective in reducing ethanol consumption. In this respect, naltrexone decreased ethanol consumption in a dose-dependent manner in a number of animal species and experimental paradigms [118-121]. Opioid receptor antagonists have been effective in the clinical treatment of patients with alcohol dependence [122-126], reducing relapse rates as well as craving and alcohol intake when combined with behavioral therapy [127, 128].

Two large meta-analytical studies [129, 130] have shown that naltrexone is efficacious in reducing the risk of relapse among recently abstinent, alcohol-dependent individuals. Importantly, the recent publication of the results of the NIAAA-sponsored COMBINE study (N = 1383) underscores the finding that naltrexone (100 mg/day) plus pharmacologic management to enhance compliance reduced the risk of a heavy drinking day compared with placebo [131]. Several functional brain imaging studies conducted during alcohol cue presentation show that naltrexone decreases alcohol cue-induced activation of the ventral striatum [132-134]. These results suggest that naltrexone relieves alcohol craving, probably by modulating DA neurons in the ventral striatum.

In relation to the long-acting effect of naltrexone, three extended-release formulations of naltrexone for deep intramuscular injection have been developed (Vivitrol®, Naltrel®, and Depotrex®). The results obtained with these formulations are inconsistent. In a large, placebo-controlled, double-blind, randomized, multi-site, 24-week clinical trial, Garbutt *et al.* [135] found that high-dose Vivitrex® (380 mg) recipients had a significantly lower percentage of heavy drinking days than the subjects who received placebo. Recipients of low-dose Vivitrex® (190 mg) had outcomes similar to those who received placebo. The treatment response signal in high-dose Vivitrex® recipients was elicited only in male participants, as the effect of the two Vivitrex® doses did not differ from that of placebo in women. The inefficacy of Vivitrol® in women is attributed to the presence of more subclinical affective symptoms, less family history of alcoholism, more responsiveness to placebo, and more clinical heterogeneity in the sample. Kranzler *et al.* [136] studied the safety and efficacy of Naltrel® in treating male and female alcohol-dependent subjects receiving monthly motivation enhancement-based therapy in a double-blind, placebo-controlled, 3-month, randomized controlled trial (N = 157). The initial dose of Naltrel® (150 mg) was delivered by deep intramuscular injection into each buttock; subsequent monthly doses were 150 mg. Placebo injections of excipient without active compound were given with the same frequency. Naltrel® was superior to placebo in increasing the mean number of cumulative abstinent days and prolonging the median time to first drink. The effect of gender on treatment outcome was not examined. A single-site, 6-week trial of 16 alcohol-dependent individuals who received one intramuscular dose of Naltrel® (300 mg) [137] suggests, somewhat paradoxically, low tolerability. Drinking outcomes showed a trend toward improvement over the course of the trial. In summary, depot naltrexone formulations may have some advantages over oral formulations, such as increased compliance. This advantage has been difficult to demonstrate in randomized controlled trials, but might become more apparent when depot formulations are used in general practice.

The opioid receptor antagonist nalmefene (6-methylene analog of naltrexone) has also been found to be effective in decreasing relapses to heavy drinking in humans [138]. Interestingly, nalmefene also produces more HPA axis activation than naltrexone [139].

Although most pharmacologic studies have focused on the modulation of μ-opioid receptors by antagonists like naltrexone, other strategies have been proposed. In first place, down-regulation of the μ-opioid receptor by RNA interference in VTA is effective in reducing ethanol consumption in mice [140]. In second place, distinct opioid receptors and their pharmacologic response have been manipulated to reduce ethanol intake. Using this second strategy, Higley *et al.* showed that δ-opioid antagonism reduces ethanol taste reactivity and ethanol consumption in outbred male rats [141]. Furthermore, intra-VTA microinjection of the δ-opioid receptor agonist DPDPE ([D-Pen^2,D-Pen^5]-enkephalin) decreased ethanol consumption in rats, particularly in low-drinking animals [142]. In addition, there is pharmacologic evidence of a motivational role of κ-opioid systems in ethanol dependence [143].

On the other hand, several authors suggest the need for combined drug therapies in the treatment of alcoholism to enhance beneficial effects and curtail negative side effects. Thus, a study that combined acamprosate with naltrexone had more beneficial effects than naltrexone alone in reducing alcohol consumption [144] and recovering alcohol-dependent patients [131, 145]. Almost subeffective doses of the NMDA receptor antagonist memantine combined with low doses of naltrexone blocked ethanol consumption in rats, suggesting that this combination may have therapeutic value in the treatment of alcoholism, especially in patients with adverse sensitivity to naltrexone [146]. Overall, these data indicate that several components of the opioid system may regulate alcohol consumption.

6. CANNABINOID PHARMACOLOGY IN ALCOHOL CONSUMPTION

Several studies suggest that stimulation of the endogenous cannabinoid system by CB1 receptor agonists enhances ethanol intake in animal models [56, 105, 106], whereas blockade by CB1 receptor antagonists produces the opposite effect. This issue has been widely explored using the CB1 receptor antagonist rimonabant (SR-141716A) [147]. For instance, rimonabant suppressed voluntary ethanol intake under the two-bottle choice paradigm in C57BL/6 mice [14, 56], Wistar rats [148], and selectively bred sP rats [15, 149, 150]. Furthermore, rimonabant repressed ethanol self-administration in Long Evans [151] and sP rats [152], and ethanol-seeking behavior in Wistar rats [153]. Lower doses of rimonabant (2.5 mg/kg) were usually more effective in reducing ethanol intake than food intake, whereas higher doses (10 mg/kg) decreased food and ethanol intake in a similar manner in ethanol-preferring Warsaw high-preferring rats [154]. Interestingly, rimonabant administration during alcoholization increases ethanol preference, whereas rimonabant given after alcoholization reduces ethanol preference. These opposing effects may be due either to increased GABA inhibition by CB1 receptor blockade during alcoholization [148] or to the inverse agonist properties of rimonabant [155]. Rimonabant blocked the development of rapid ethanol tolerance in Wistar rats, whereas the CB1 receptor agonist WIN 55,212-2 facilitated rapid tolerance [156]. Recently, Soyka *et al.* [157] conducted a controlled study for assessing the efficacy of rimonabant in the treatment of alcohol-dependent subjects. The results indicated that rimonabant 20 mg/d in recently detoxified alcohol-dependent patients does not significantly increase the time to the first drink compared to placebo. The

difference (8% better outcome) was more marked in patients who relapsed to "heavy drinking." The results of this study do not rule out a role of CB1 antagonists in the treatment of alcohol dependence. The lack of efficacy in this study may be due to a high response rate and relatively short treatment duration in the placebo group.

Another CB1 receptor antagonist (SR-147778) reduced ethanol preference in Wistar rats when administered at the cessation of chronic pulmonary ethanol intoxication, although a high dose of SR-147778 during alcoholization induced an increase in ethanol preference at the beginning of the free-choice period [158]. This may be due to interference of high-dose SR-147778 with appetitive behavior. On the other hand, acute administration of the cannabinoid CB1 receptor agonist CP-55940 increased ethanol preference in Wistar rats [105] and in ethanol-preferring C57BL/6J mice [56]. Furthermore, the CB1 receptor agonist WIN 55,212-2 promoted voluntary ethanol intake in ethanol-preferring Sardinian sP rats [106]. Other therapeutic strategies are based on the fact that increasing anandamide levels with FAAH inhibitors or FAAH gene deletion can activate the endocannabinoid system. In fact, FAAH knockout mice showed an increase in ethanol preference, and wild-type littermates treated with the selective FAAH inhibitor URB597 showed a marked increase in ethanol preference. Moreover, in the same study, URB597 had no effect on FAAH and CB1 knockout mice [159]. Alternatively, activation of the endocannabinoid anandamide system by URB597 in Sardinian ethanol-preferring rats did not increase excessive ethanol consumption, but did reduce anxiety associated with ethanol withdrawal [112]. Recently, it has been proposed that the cannabinoid CB1 receptor might be activated by the increase in endocannabinoids induced by administration of the anandamide transporter inhibitor AM404. AM404 significantly and selectively reduced ethanol self-administration in rats, although this effect was not reversed by either the cannabinoid CB1 receptor antagonist rimonabant or the cannabinoid CB2 receptor antagonist AM630 [160].

Overall, these reports suggest that CB1 receptor antagonists may be a novel alternative for the therapy of alcohol use disorders. Research continues into other endocannabinoid system components that may alleviate alcohol-related problems.

7. OPIOID AND CANNABINOID GENETIC FEATURES IN ALCOHOLISM

One major concern in the design of previous studies related to the opioid system was whether the reported abnormalities were biological markers of an underlying genetic predisposition to alcoholism or a consequence of chronic alcohol exposure. To examine the etiologic significance of the endogenous opioid system in alcoholism, Wand *et al.* [161-164] conducted a series of studies to compare HPA axis responses to opioid blockade in subjects from families with a high prevalence of alcohol dependent members and in subjects from families with no history of alcoholism. The findings of these studies suggest that non-alcoholic offspring of families with a history of alcoholism have altered cortisol responses to naloxone administration [161, 163]. Offspring at high risk for excessive alcohol consumption by virtue of their family history may have an inherited or acquired deficiency in the activity of the endogenous opioid system. These deficits may be the result of less synaptic opioid content, reduced opioid receptor density, and/or differences in the type or binding affinities of opioid receptors in specific brain regions. Furthermore, it appears that positive familial loading for alcoholism might predict the potential anti-drinking and anti-craving effects of naltrexone in human studies. King *et al.* [165] showed that social drinkers with familial loading for alcoholism were more likely to experience diminished alcohol stimulant effects following naltrexone treatment than social drinkers without familial loading for alcoholism. Recent studies suggest that naltrexone increases the urge to drink among alcohol-dependent subjects who are aspartate (Asp) carriers of the OPRM1 gene but has no effect on their homozygotic, *i.e.* asparagine-carrying, counterparts in a cue-reactivity laboratory paradigm [166]. These results are discrepant with the report showing that naltrexone preferentially protected against relapse in Asp-carrying alcohol-dependent individuals [167]. Furthermore, a recent clinical trial proposed that naltrexone treatment does not have a preferential effect on any of the variants of the OPRM1 gene [168]. The G118 allele might be associated with lower OPRM1 protein expression than the A118 allele [68], although its frequency may vary considerably between populations. Taken together, these results suggest than more molecular genetic studies are needed to further elucidate the role of the Asp40 allele and to establish whether or not naltrexone response differs in relation to variations of the OPRM1 gene.

Another problem related with genetic association studies is that disorders with a behavioral component, such as alcoholism, are phenotypically variable and likely to be influenced by multiple genes, with each gene carrying only a small variation in the phenotype [62, 169]. In addition, some genetic traits related to a specific population, for instance, Pro129Thr polymorphism in the FAAH gene associated with alcohol abuse and dependence in a Caucasian population, could not be extrapolated to a large Japanese group of people with alcoholism [170].

8. CONCLUDING REMARKS

In recent years, several reports have suggested an interaction between the endogenous opioid and endocannabinoid systems. Activation of cannabinoid receptors stimulates the release of endogenous opioid peptides and activates opioid receptors [22, 23]. Moreover, opioid receptor antagonists partially block some effects of Δ^9-tetrahydrocannabinol that are related to alterations in the reward system [171]. Opioid antagonists, such as naloxone (administered systemically) or naloxonazine (infused into the ventral tegmentum), prevent the action of cannabinoids and heroin on dopamine transmission [172]. Several studies in rats suggest that chronic administration of cannabinoid agonists influences the opioid system by increasing POMC gene expression in the arcuate nucleus of the hypothalamus [22] and proenkephalin gene expression in the periaqueductal gray matter, NAcc, paraventricular and ventromedial hypothalamic nuclei, and medial mammillary body [173], and up-regulating μ-opioid receptor function [174] (Table **1**).

Table 1. Effects of Cannabinoids on the Opioid System

Cannabinoid Ligand	Effects on opioid system		
	Gene	Region	Refs.
Δ^9-THC	↑ POMC	Arc	[22, 23]
	↑ PDYN	spinal cord	
	↑ PENK	spinal cord	
	↑ μ- opiod receptor	CPu	[169]
	↑ PENK	VMN, PAG, Mammilary nucleus	[168]
	= PENK	CPu, NAcc	
AM356	↑ PENK	VMN, PAG, Mammilary nucleus	[168]
	= PENK	CPu, NAcc	
CP-55,940	↑ PENK	CPu, NAcc, PVN,VMN	[168]

Δ^9-THC: Delta 9-tetrahydrocannabinol; AM356: R-methanandamide; CP-55,940: CB1 agonist; POMC: Pro-opiomelanocortin opioid peptide; PDYN: prodynorphin; PENK: proenkephalin; Arc: Arcuate nucleus; CPu: Caudate putamen; VMN: Ventromedial hypothalamic nucleus; PAG: Periaqueductal gray; NAcc: Nucleus accumbens; PVN: Paraventricular nucleus.

In addition, the blockade of cannabinoid CB1 receptors may impede the ethanol-induced increase in opioid release. Therefore, cannabinoid receptor antagonists presumably may alter opioid peptide release and decrease alcohol consumption. In contrast, the administration of cannabinoid receptor agonists enhances endogenous opioid activity [22-24]. These findings suggest that differences in endogenous cannabinoid and opioid function may be responsible for a distinct vulnerability to alcohol consumption and/or dependence. Furthermore, the increase of ethanol consumption induced by the cannabinoid receptor agonist CP-55940 or WIN 55,212-2 may be blocked by administration of either naltrexone or SR-141716A [24].

The fact that both opioid and cannabinoid antagonists tend to normalize opioid function, which is disrupted by ethanol intake, suggests a potentially synergistic action to reduce ethanol consumption. Thus, combined low-dose treatment with opioid and cannabinoid receptor antagonists synergistically reduces the motivation to consume ethanol in rats [175]. In general, these results suggest a shared role of the cannabinoid and opioid receptor systems in the management of alcohol intake and craving, and new and potentially useful therapeutic strategies for alcoholism may emerge from the combination of cannabinoid and opioid receptor antagonists [152].

The narrow relationship between these two endogenous systems may shed light on our comprehension of the molecular mechanisms involved in alcohol dependence. Clinical practices derived from therapeutically manipulated cannabinoid/opioid crosstalk have yet to be developed, but knowledge of this interaction could result in new therapeutic options for alcohol use disorders.

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