Role of the Endocannabinoid System in Alcohol-Related Behaviors

Basalingappa L. Hungund[*,1,2,3] and K. Vinod Yaragudri[*,1,2,4]

[1]*New York State Psychiatric Institute, New York, USA*

[2]*Division of Analytical Psychopharmacology, Nathan Kline Institute for Psychiatric Research, Orangeburg, New York, USA*

[3]*Division of Analytical Psychopharmacology, Department of Psychiatry, College of Physicians & Surgeons, Columbia University, New York, USA*

[4]*Department of Child & Adolescent Psychiatry, New York University School of Medicine, New York, USA*

Abstract: Alcoholism is a psychiatric disorder characterized by impaired control over drinking, leading to tolerance, physical dependence, uncontrollable craving and relapse. The mechanism/s underlying this disorder is poorly understood at present. Ethanol (alcohol) effects are mediated through several signal transduction pathways involving many neurotransmitters and ion channels in various brain regions. There is a growing body of evidence now suggesting a critical role for the endocannabinoid (EC) system in alcohol-related behaviors. The EC system is comprised of endogenous cannabimimetic substances (endocannabinoids) and their receptors [cannabinoid (CB)] and the enzymes involved in the synthesis and degradation of the ECs. Recent studies have demonstrated that both the genetic and pharmacological manipulation of the EC system modulate the development of tolerance to and dependence on alcohol. The present article provides a review of the existing literature on the role of the EC system, and possible mechanisms and the therapeutic potential of the drugs targeted against this system in preventing alcohol addiction.

Keywords: Alcoholism, anandamide, CB1 receptor, dopamine, nucleus accumbens.

INTRODUCTION

For centuries marijuana has been used both recreationally and for the treatment of a variety of illnesses that include glaucoma, nausea, vomiting, pain management and many other illnesses [1]. However, because of the psychoactive side effects of tetrahydrocannabinol (THC), the active ingredient of marijuana, its use for medical purposes has been limited. During the late sixties and early seventies, research efforts were directed towards understanding the neurobiological mechanisms underlying the pharmacological effects of THC. Following the cloning of the CB1 receptor in 1990 [2] and the discovery of the EC, arachidonyl ethanolamide (AEA; anandamide) in 1992 [3], there has been a significant surge in research activities related to behavioral and pharmacological effects of THC. The EC system is now implicated in many aspects of health and diseases. The drugs targeted against this system have potential therapeutic utility in the treatment of depression, anxiety, obesity, diabetes and drug addiction. This article summarizes the current knowledge of the role played by the EC system in a number of alcohol-related behaviors.

THE ENDOCANNABINOID SYSTEM

The EC system is comprised of cannabinoid receptors, their endogenous ligands and the enzymes involved in their synthesis and degradation [4-9]. To date two subtypes of G-protein coupled receptors (GPCRs) have been cloned; the cannabinoid CB1 and CB2 [9]. It was originally thought that the CB1 receptors are exclusively localized in the central nervous system (CNS). However, recent studies suggest that they are also found in the peripheral system. The CB2 receptors which were thought to be restricted to peripheral system have now been shown to be present in the CNS [10]. The CB1 receptors are among the most abundant neuromodulatory GPCRs in the CNS and are comparable to those of other aminergic receptors [9, 11, 12]. These receptors are coupled negatively to adenylate cyclase (AC) and N- and P/Q type Ca^{2+} channels and positively to A-Type and inwardly rectifying K^+ channels and mitogen-activated protein kinases (MAPKs) through G_i/G_o proteins [9]. The CB1 receptors are highly expressed in the cortex, hippocampus, cerebellum and basal ganglia [9, 11, 12] and mediate many of the actions of known neurotransmitters and hormones. The neuroanatomical and electrophysiological studies suggest that the CB1 receptors are localized in the presynaptic terminals of the neurons [13, 14]. Evidence for the existence of a third type of cannabinoid receptor is also emerging [7]; however the characterization of this receptor is incomplete at the present time.

Several types of endogenous agonists for the CB receptor have now been reported to exist in the mammalian CNS. Most of these compounds are derived from arachidonic acid and among them AEA and 2-arachidonyl glycerol (2-AG) have been most studied [3, 15, 16]. Unlike other classical neurotransmitters, the ECs are not stored in the vesicles, but are released upon demand from membrane lipids triggered by enhancement of intracellular calcium [17-19]. A major

*Address correspondence to these authors at the Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY 10962, USA; Tel: 845-398-5452; Fax: 845-398-5451;
E-mails: hungund@nki.rfmh.org and vyaragudri@nki.rfmh.org

1876-5238/09

pathway for the synthesis of AEA is suggested to be by catalysis of its precursor, N-arachidonyl phosphatidyl ethanolamine (NAPE) by NAPE specific PLD (NAPE-PLD) [20]. On the other hand, 2-AG has been shown to be derived from the hydrolysis of 2-arachidonate containing diacyl glycerol (DAG) *via* DAG lipases [5, 19]. Although controversial, there have been suggestions that both AEA and 2-AG are transported across the cell membrane *via* membrane transporter proteins [21-24]. The AEA is inactivated by the fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine [21, 23, 24] and monoacyl glycerol lipase is mainly involved in the degradation of 2-AG [25-26].

Higher levels of the CB1 receptors are found in the basal ganglia and other reward-related brain regions [27-29]. Immunocytochemical studies have also demonstrated that the FAAH enzyme is co-localized with the CB1 receptors [30, 31]. It has been suggested that the ECs are synthesized in the postsynaptic neurons and are released into the synaptic cleft, where they act as retrograde messengers [14]. They are removed rapidly from the extracellular space through uptake and catabolism [17, 32-34]. In the CNS, the ECs activate CB1 receptors and regulate synaptic transmission of both excitatory and inhibitory circuits by modulating the release of monoamine neurotransmitters [9, 14].

ALCOHOLISM AND ENDOCANNABINOID SYSTEM

Intensive research efforts are currently being directed towards answering the question as to what observed changes in the brain function can adequately explain the behavioral effects produced by alcohol. Although the exact mechanism of action of alcohol with its neuronal targets is not well understood, there is evidence to suggest that alcohol interacts with specific neuronal membrane proteins to alter their normal function [35]. These interactions perturb the intra and intercellular signaling systems, thereby exerting diverse and profound effects on neural responses. For example, there is evidence that supports a direct interaction of alcohol with GABA-A, glutamate, and serotonin receptors [35]. Recent studies from our laboratory and others have implicated the EC system in many of the alcohol-related behaviors. The following section reviews the current literature on the role on the EC system in alcohol tolerance, dependence and voluntary alcohol consumption.

Effect of alcohol on the EC system: The CB1 receptors are localized in many of the brain regions such as the cortex, striatum, substantia nigra, hypothalamus, hippocampus and cerebellum. The neuroanatomical distribution of the CB1 receptor is also consistent with the behavioral effects produced by alcohol that include decreased motor activity, hypothermia, memory consolidation and catalepsy [7, 36, 37].

The up-regulation of AEA and a simultaneous down-regulation of NAPE have been shown to occur in the brain of mice that were chronically exposed to alcohol [38, 39]. *In vitro* studies have revealed an elevation in the levels of AEA and 2-AG in chronic alcohol exposed SK-N-SH cells and granular neurons [39, 40]. It is interesting to note that chronic alcohol exposure also increases the levels of ECs (AEA and 2-AG) in the "limbic" forebrain [41], a key brain region implicated in reinforcing properties of addictive drugs. This increase appears to be associated with activation of calcium-dependent and AA-specific PLA2, a key enzyme involved in the formation of ECs [42]. The content of these ECs decrease following 24-48h of alcohol withdrawal [41, 43, 44], which is associated with the up-regulation of CB1 receptors [44], suggesting a neuroadaptation in the EC system during alcohol abstinence. On the other hand, acute alcohol treatment has been shown to reduce the AEA levels in the brain without affecting the FAAH activity [45-47], indicating a reduction in the endocannabinoid-mediated signaling by acute alcohol exposure.

The receptor activation of G-protein is a key step in signal transduction pathway that is found to be altered in response to exposure of many drugs of abuse. Previous studies have shown a down-regulation of the CB1 receptor and CB1 receptor-stimulated G-protein activation in the brain of mice following exposure to alcohol [44, 48-50]. Consistent with these findings, a reduction in gene expression of the CB1 receptor has been reported in caudate putamen (CPu), ventromedial nucleus of the hypothalamus (VMN), and hippocampus of rats exposed to chronic alcohol [51]. Chronic intermittent exposure of alcohol and its withdrawal also transiently down-regulates hippocampal CB1 receptors followed by a long-term up-regulation, including elevation in the ECs levels [52]. However, Gonzales *et al*. (2002) did not observe any significant change in the CB1 receptor density in the brain regions of rats subjected to voluntary alcohol consumption [53]. These contradictory findings may possibly be due to differences in alcohol exposure paradigm. It is possible that the regional differences in adaptation may vary depending on the amount and duration of alcohol exposure.

The mechanism by which chronic alcohol decreases the expression of CB1 receptors is not clearly known at this time. However, it is generally accepted that GPCRs undergo desensitization when continuously stimulated by their endogenous agonists. Thus, the desensitization of CB1 receptor-mediated signaling by chronic alcohol may be the result of overstimulation of the CB1 receptors by AEA, which is increased in response to chronic alcohol exposure [38-41, 44]. Alternatively, chronic alcohol may inhibit AEA transporter resulting in an increase in the levels of AEA [22]. An elevation in the AEA level by chronic alcohol exposure seems to be due to reduction in the activity of FAAH enzyme [44]. Taken together, these changes in the EC system may represent a neuroadaptation to chronic alcohol exposure and may be associated with the development of tolerance to and dependence on alcohol.

Role of the EC signaling in alcohol tolerance and dependence: It is well established that repeated exposure to alcohol leads to the development of tolerance and dependence and this process involves several neuroadaptive changes in the brain. There are several studies which have attempted to investigate whether genetic or pharmacological manipulation of CB1 receptor function [54, 55] and FAAH enzyme [56] could alter development of tolerance and dependence to alcohol. For instance, the CB1 receptor gene deleted mice (CB1-KO) mice, which are known to drink less alcohol, exhibit a greater sensitivity to alcohol [54].

An acute dose of CB1 receptor agonist, WIN 55, 212-2 is shown to facilitate the development of rapid tolerance to

alcohol and is blocked by SR141716A (CB1 receptor antagonist) when Wistar rats are subjected to motor-coordination test [57]. The effect of CP-55, 940 appears to vary depending on the behavioral parameter tested. The development of tolerance to acute alcohol-induced hypothermia depends on the timing of the administration of the agonist. For example, tolerance occurs when the CB1 receptor agonist is given prior or along with alcohol treatment [58]. However, chronic blockade of CB1 receptor with SR141716A results in tolerance development to both sedative and hypothermic effects of alcohol in alcohol naïve mice without any effect in chronic alcohol exposed mice [58]. It is not clear at this time as to what the mechanisms are to explain these varying effects of the agonist and the antagonist on tolerance. However, it should be noted that both the THC and AEA are cross tolerant to alcohol and vice versa suggesting a possible common mechanism for development of tolerance/dependence for these drugs involving the EC system.

The alcohol withdrawal symptom is a major problem associated with relapse to alcohol dependence. A number of studies have implicated a role for the EC system in alcohol dependence and relapse. Pharmacological manipulation of the CB1 receptor function with SR141716A has been shown to block the alcohol deprivation effect (temporary increase in alcohol intake after a period of alcohol withdrawal) in alcohol-preferring Sardinian rats (sP) [59]. The antagonism of the CB1 receptor function also attenuates the behavioral symptoms elicited after a 3h interruption of chronic alcohol exposure [60]. In addition, SR141716A has been found to correct the imbalance in GABA and glutamate in the brain regions involved in emotional and motor functions and dopamine (DA) deficits in reward-related brain regions caused by alcohol withdrawal [60]. A noncontingent chronic exposure to WIN 55212-2 during alcohol deprivation has been shown to potentiate the relapse to alcohol use suggesting that functional changes in the CB1 receptor may play a key role in relapse to alcohol [61]. Furthermore, the alcohol withdrawal symptoms as measured by handling-induced convulsions (HIC) were also found to be reduced in C57BL/6J mice lacking CB1 receptor gene [54, 62]. However, mutant mice generated on CD1 background exhibited higher sensitivity to acute alcohol and increased withdrawal severity after chronic alcohol exposure [54]. This discrepancy could possibly be due to differences in the genetic makeup of the two strains. There is some clinical evidence indicating the involvement of the EC system in alcohol dependence. For instance, a recent report correlated the severity of withdrawal symptoms with CB1 gene polymorphism [63].

There are few studies that have explored the role of FAAH in alcohol dependence. The severity of HIC is found to be lower in FAAH-KO than WT mice [56]. Conversely, Blednov *et al*., (2007) reported no difference in the HIC in these mice [64]. The possible explanation for this inconsistency might be due to differences in alcohol dosage administered (chronic versus acute). One possible mechanism by which AEA produces such changes might be through interactions with inhibitory and excitatory neurotransmissions. The physical signs of withdrawal are primarily thought to be caused by imbalance between inhibitory GABA and excitatory glutamatergic neurotransmitter systems [65]. In addition, chronic alcohol exposure increases AEA in the brain [39, 40, 44, 52] that could reduce the uptake of GABA [66, 67] resulting in enhanced inhibitory neurotransmission. These interactions might reduce the severity of HIC in FAAH-KO mice. The activation of presynaptic CB1 receptors that are present on GABAergic and glutamatergic interneurons through ECs, such as AEA, also appear to play a critical role in regulating inhibitory and excitatory neurotransmissions [68, 69]. The AEA has been found to modulate the activity of NMDA receptor *via* non-CB1 receptor mediation [70] and has been shown to antagonize cocaine-induced lethality and NMDA-induced convulsive seizures [71]. The pretreatment with SR141716A abolishes anticonvulsant effect of AEA in an electroshock seizure model, indicating the modulation of seizure activity by AEA tone. The pretreatment with the blocker of AEA transporter, N-(4-hydroxyphenyl) arachidonoylamide (AM404) also prevents the opioid receptor antagonist (naloxone)-induced seizures [72]. In addition, an inverse genetic relationship between alcohol self-administration and withdrawal severity has been reported [73]. These findings demonstrate that AEA has anticonvulsant activity and further implicate the CB1 receptor to be one of the major targets of seizure modulation [74] and further suggest that the drugs targeted against the components of the EC signaling system may have therapeutic utility in the treatment of alcohol dependence and withdrawal effects.

Manipulation of CB1 receptor function on voluntary alcohol consumption: Recent reports provide evidence for a direct linkage between CB1 receptor function and alcohol consumption [53, 54, 75-89] (Table **1**). For instance, the administration of SR141716A (rimonabant) reduces alcohol consumption in rodent models [54, 75-82] (Fig. **1**). A combined low dose of opioid and CB1 receptor antagonists also synergistically decreases motivation to drink alcohol [78]. Acute administration of rimonabant also suppresses operant alcohol self-administration in Wistar rats [79]. While concurrent administration of rimonabant with chronic alcoholization increases the preference for alcohol [80, 81], its administration after the chronic alcoholization or at the time of withdrawal drastically diminishes the alcohol preference [81]. Interestingly, the prefrontal cortex (PFC) appears to play a critical role in regulating the alcohol drinking behavior as revealed by a dose dependent suppression of alcohol self-administration in alcohol preferring rats when rimonabant was given locally into the PFC [82]. Conversely, the CB1 receptor stimulation is shown to enhance alcohol drinking behavior in both alcohol preferring and alcohol non-preferring rodents [55, 61, 84, 104]. In addition, an increased motivation to drink more beer in rats following administration of the CB1 receptor agonist (CP-55, 940) and complete abolition of this effect after treatment with rimonabant has also been shown [83].

Newly developed methods for targeted alteration of gene function offer the possibility of establishing a cause and effect relationship between molecular targets of alcohol action and alcohol-induced behavioral changes. In this regard, generation of CB1 receptor null mutant mice has provided several insights into the role of CB1 receptor in physiology and behavior. It has been demonstrated that mice lacking CB1 receptor gene with two different genetic background (C57BL/6J and CD1) consume less alcohol

Table 1. Modulation of Alcohol Drinking Behavior by the EC System

Approach	Finding	Animal Model	References
CB1 deletion	↓	Mice	[54, 55, 84-89]
SR141716A	↓	Rats, Mice	[54, 75-82, 88]
CP55, 940	↑	Rats, Mice	[55, 83, 104]
WIN55, 212-2	↑	Rats, Mice	[61, 84, 104]
FAAH deletion	↑	Mice	[56, 64, 92]
URB597	↑	Rats, Mice	[56, 64, 82]
URB597	↔	Rat	[93]

Effect of genetic and pharmacological manipulation of CB1 receptor and FAAH activities on alcohol drinking behavior in animal models. ↑ Increase; ↓ Decrease; ↔ No effect.

[54, 55, 84-89] (Fig. **1**) and exhibit reduced effect on alcohol-induced DA release in the nucleus accumbens (NAc) compared to their WT controls [85]. A reduction in conditioned place preference and increased striatal D2 receptors has also been reported in CB1-KO mice [87-89]. Moreover, the genetically determined changes in the activities of components of the EC system in alcohol preferring and alcohol avoiding animals might also explain differences in alcohol drinking behaviors [55, 82, 90]. These studies strongly support a role for the CB1 receptor in alcohol drinking behavior.

Role of the FAAH in alcohol-related behavior: Since the agonists and the antagonists of the CB1 receptors modulate alcohol-reinforced behavior, the obvious question to ask is whether the genetic or pharmacological manipulation of the FAAH, a key enzyme that is responsible for regulating the brain AEA [91], would influence alcohol consummatory behavior. There are a limited number of studies that have investigated the role of FAAH in alcohol drinking behavior [55, 64, 92]. It was reported that the mice lacking FAAH gene on a mixed genetic background (B6/129SV/J) consume significantly more alcohol compared to their WT counterparts [92]. An increase in alcohol intake has been shown in mice following administration of the FAAH inhibitor URB597 as well as in genetically homogeneous B6 mice lacking FAAH gene [55, 64] (Fig. **2**). Conversely, recent study reported no effect of URB597 on alcohol intake in Wistar rats [93]. Further support for the participation of FAAH in alcohol drinking-related behavior is derived from a comparison study of the expression of the EC-related genes in alcohol-preferring and alcohol non-preferring rats, in which a decrease in the expression of FAAH activity in the PFC of alcohol-preferring rats has been observed [82]. An association of an impaired FAAH activity with alcohol self-administration is further supported by an increased alcohol self-administration in Wistar rats, which were given intra-PFC injection of URB597 [82]. Furthermore, the administration of AEA alone or URB597, has been shown to increase the DA levels in NAc shell suggesting that AEA through the activation of the mesolimbic dopaminergic system, may produce rewarding effects [94]. Nevertheless, an increased vulnerability to drug and alcohol abuse in humans has recently been suggested to be due to polymorphism in the FAAH gene and reduced FAAH expression and activity [95, 96]. Furthermore, the FAAH-KO mice exhibit decreased sensitivity to alcohol-induced hypothermia, sedation and locomotor effects and reduction in alcohol withdrawal convulsions [55]. A faster recovery from motor incoordination following acute alcohol administration was also found in FAAH-KO mice [64]. This lower response appears to be one of the physiological factors that could be attributed to a greater alcohol drinking behavior in FAAH-KO mice. It is inferred from these studies that the impaired FAAH function that leads to increased brain levels of AEA may confer a phenotype of high voluntary alcohol intake, and suggests FAAH to be both as a potential susceptibility factor and a therapeutic target.

NEUROBIOLOGICAL BASIS OF ALCOHOL ADDICTION

To understand the neurobiological basis of alcohol addiction, it is critical to know the neural circuitries implicated in initiation/maintenance of addiction. In this regard, the dopaminergic neurotransmitter system in the prefrontal and ventral striatal regions have long been implicated in reward circuitry. The mesolimbic dopaminergic system mainly consists of dopaminergic neurons whose cell bodies are located in the

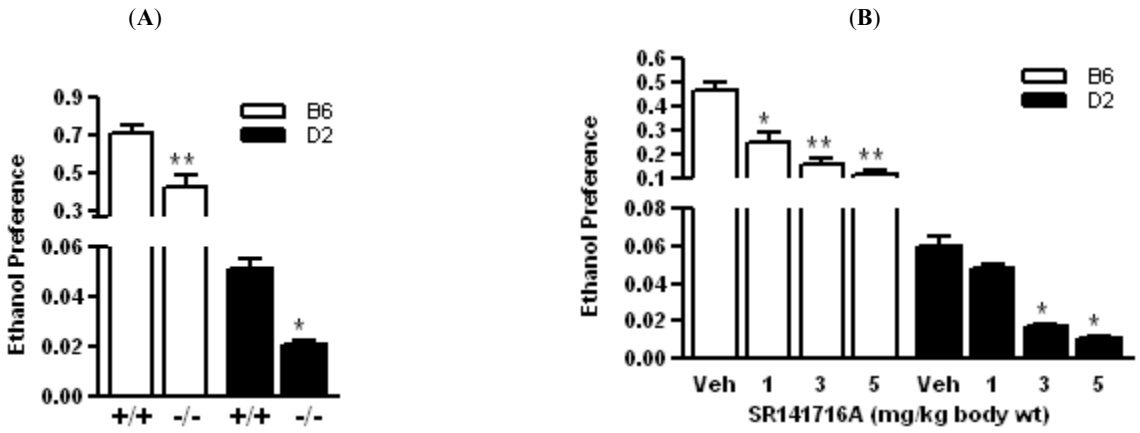

Fig. (1). In an unlimited access paradigm, the preference for ethanol was found to be significantly lower in B6.CB1 -/- ($p<0.001$) and D2.CB1 -/- ($p<0.05$) compared to their corresponding +/+ mice (**A**). In a limited access paradigm, the CB1 receptor antagonist, SR141716A, reduced ethanol preference in B6 and D2 strains compared to vehicle treated groups (**B**). (Adapted from Vinod *et al*., 2008).

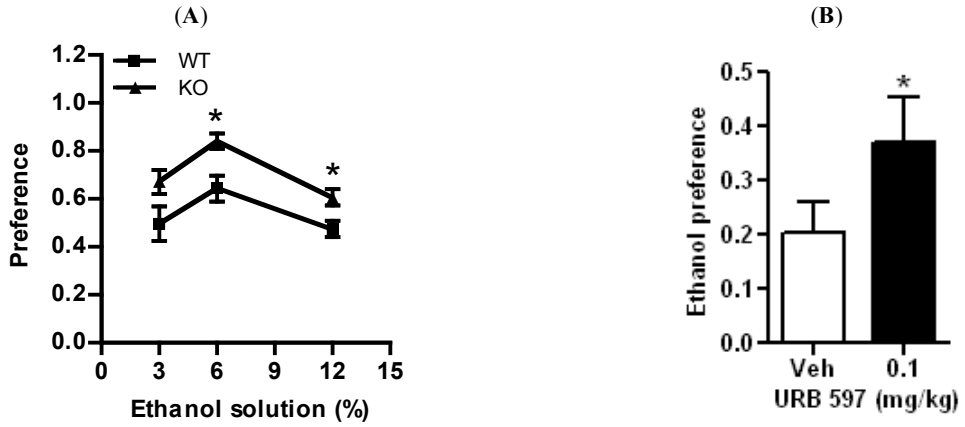

Fig. (2). The mice lacking FAAH gene (FAAH-KO) displayed a greater preference for 6% (35%, $p<0.01$) and 12% ethanol solutions (26%, $p<0.05$) compared to WT mice (**A**). In a limited access paradigm, FAAH inhibitor, URB597 (0.1 mg/kg), significantly enhanced the preference for 12% ethanol solution (100%, $p<0.01$, **B**) in C57BL/6J mice than vehicle treated control group. (Adapted from Vinod *et al*., 2008).

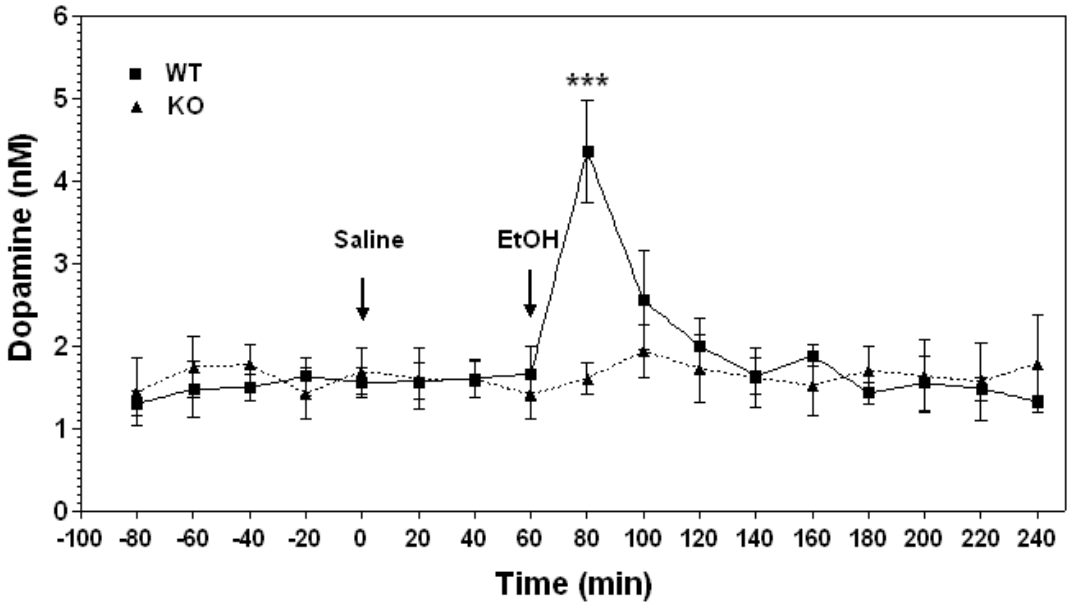

Fig. (3). Alcohol (EtOH) induced Dopamine release in the nucleus accumbens was significantly (***$p<0.001$) reduced in CB1 KO compared to wild type (WT) mice. (Adapted from Hungund *et al*., 2003).

ventral tegmental area and project terminals into the NAc, frontal cortex, amygdala, and septal area [97], has been implicated in mediating the reinforcing and self-administration mechanisms of various drugs of abuse, including alcohol. Alcohol has been shown to acutely increase extracellular DA levels in nucleus accumbens (NAc), which may increase hedonic experience and thus accumbal DA may play a role in the development of addiction to alcohol. As discussed earlier, the components of the EC system do seem to contribute to this effect. The pharmacological blockade as well as deletion of CB1 receptor gene reduces the acute alcohol-induced release of DA in the NAc in mice [85] (Fig. **3**). Furthermore, alcohol consumption has been shown to increase AEA content in limbic forebrain [41], which appears to activate mesolimbic dopaminergic transmission by increasing the DA release in NAc. In this regard, an intravenous administration of both AEA and methanandamide (a stable derivative of AEA) and pharmacological inhibition of FAAH with URB597 that enhances the brain levels of AEA, has been shown to increase accumbal DA [94] preferably by activation of the CB1 receptor *via* ventral tegmental area; whereas the antagonism of the CB1 receptor reduces the DA release in the NAc [98]. The persistent drug use might be associated with repeated activation of mesolimbic DA system, which could enhance incentive value of the drug of abuse. These drug-induced changes in the accumbal DA function have been hypothesized to lead to the progression from reward to addiction. Thus, the alcohol-induced DA release in the NAc that is mediated by the EC system might provide one of the mechanistic explanations for the pathophysiology of alcohol addiction (Fig. **4**).

It is also important to consider other neurotransmitter systems such as excitatory and inhibitory systems that may be highly relevant in drug addiction, which are widely distributed throughout the brain. Converging lines of evidence suggest a role for the CB1 receptor in modulation of several other neural circuits involved in reinforcement,

alcohol addiction involving the EC system before the appropriate molecular target/s can be determined.

ACKNOWLEDGEMENTS

The support for our studies were provided by NIH grants N01AA2208; AA13003. We are thankful to Dr. Henry Sershen for his helpful comments in preparing this manuscript.

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Received: July 1, 2009 Revised: July 20, 2009 Accepted: July 21, 2009