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2nd DRAFT
STUDENT ESSAY

Roles of Endocannabinoid System in Alcohol Abuse

Introduction

Alcohol has always caused significant health issues in our society. According to the World Health Organization in 2000, alcohol was responsible for 4% of global disease. Ethanol is toxic to the cardiovascular system, digestive system, central nervous system, peripheral nerves, musculoskeletal system and the fetus.

Alcoholism is one of the most common social problems. It is considered as a chronic relapsing disorder accompanying various detrimental effects, such as a negative emotional state and visceral diseases. Chronic alcohol exposure leads to the increase of intake and tolerance this can happen even before noticing intoxication.

So, how can alcoholism be prevented? What are the underlying mechanisms? Neuroadaptation, genetic risks, and metabolisms associated with alcohol abuse? One of the major systems being affected is the endocannabinoid system of the body.

Endocannabinoid system

The endocannabinoid system is a cell signalling system in the body which responds to the environment ,internally or externally, consisting of cannabinoids and cannabinoid receptors It is widely distributed in tissues including endogenous ligands, receptors, and biosynthetic and hydrolysing machineries. The endocannabinoid system has also been proven to have a strong association with various behavioural, neurological, metabolic and fertility disorders and maintenance of a healthy ecosystem.

Till this day, two cannabinoid receptors have been discovered and cloned, one is the CB1 receptor, occurring mainly in the brain, which plays an important role in the regulation of neurotransmitter release. The CB2 receptor is found mainly in the peripheral tissues, such as spleen, tonsil, thymus and lymphoid tissues.

Cannabinoids are famous, especially as a chemical substance contained in marijuana, such as delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) extracted from the plant Cannabis Sativa. Endocannabinoids refer to endogenous agonists of CB receptors, such as N-arachidonoyl-ethanolamine (AEA; anandamide) and 2-Arachidonoylglycerol (2-AG).

Interaction between alcohol and cannabis

There is a synergistic interaction between alcohol and cannabis use. While alcohol confers tolerance to cannabis treatment, cannabis enables the acute effects of alcohol. It is also found that the acute tolerance effects of alcohol are mediated through CB1 receptors (da Silva et al. 2001). Even though alcohol and cannabis have their own unique effects, there is related cognitive deficits which occur due to acute and chronic exposure.

Cannabinoid and ethanol have several biochemical and behavioural correspondences , as shown by several studies. For example, studies have shown increased endocannabinoid signalling leads to a generalized decrease in sensitivity to multiple physiological and behavioural responses for ethanol. In general terms of biochemistry there is a definitive symmetrical cross tolerance displayed while in the behavioural aspect, ethanol and cannabis may serve as a substitute for one another when treating their negative behavioural symptoms.

There is also a marked genetic aspect that needs to be taken into account in regard to these biochemical processes and behaviours induced by endocannabinoid and ethanol which can affect their correspondence which will be explained further on in the essay.

Functions of endocannabinoids in alcohol abuse disorders

Using mice, it is shown that chronic alcohol exposure decreases the number and functions of CB1 receptors in the brain (Basavarajappa & Hungund, 1999). CB1 receptor agonists increase alcohol intake, while CB1 receptor antagonists decrease it, indicating that alcohol intake is under the control of CB1 receptors (Gallate et al., 1999; Arnone et al., 1997). With the fact that CB1 receptors are present in presynaptic terminals, the endocannabinoid system, especially CB1 receptors for presynaptic effects, plays a role in modulating alcohol intake properties.

Biochemical evidence

Several experiments were done to showcase the effect of alcohol on the endocannabinoid system. Few in vitro studies were carried out. The first experiment proved that chronic ethanol intake increased anandamide* formation in SK-N-SH cells. This increase is inhibited by the antagonist of CB1 called rimonabant and pertussis toxin.

The second experiment showed the increase of 2-arachidonoylglycerol formation in cerebellar granule neurons after chronic ethanol abuse.

Anandamide and 2-arachidonoylglycerol are the most studied endocannabinoids. They are produced upon a stimulus and are released in the extracellular environment to bind to CB1 or CB2 receptors.

In ethanol treated rats, the anandamide concentration was increased in the limbic forebrain but it decreased in the midbrain. Some studies also indicate the decrease in CB1 receptors in the mouse brain. A recovery of the decreased amount of CB1 receptor binding and functionality was examined after the withdrawal of alcohol.

In postmortem human brains, those who died of suicidal alcoholic backgrounds had hyperactivity of the endocannabinoid signaling in the prefrontal cortex. They had higher CB1 receptor concentration and higher functionality, at the same time, the concentration of anandamide and 2-arachidonoylglycerol was higher. Acute alcohol administration results in reduced levels of endocannabinoids in different brain regions.

Multiple preclinical studies on rats were the first to definitely show a symmetrical cross tolerance between ethanol and endocannabinoid.

Using a one way avoidance paradigm in a study, rats were shown to display impaired avoidance behaviour when given acute administration of either THC or ethanol. A population with repeated treatment with the other compound displayed a marked tolerance and their performance impairment was greatly reduced.

Cross tolerance can arise from the ataxic effects of cannabinoids and ethanol. This tolerance was not due to pharmacokinetic processes because in studies ethanol tolerant subjects didn't metabolise THC more readily, and in kind, THC tolerant rats did not show an increased metabolism of ethanol and overall turnover.

In a study, mice treated chronically with ethanol for a duration of ten days displayed significantly reduced sensitivity to cannabinoid-induced hypomotility, hypothermia and antinociception that was correlated with changes in the CB1 receptor expression in the hypothalamus.

Although there is currently not a specific receptor associated with the mechanism of action for ethanol, a robust data set has emerged in the past 20 years that strongly indicates the majority of ethanol's actions at intoxicating concentrations are mediated by relatively weak interactions with a wide variety of molecular targets that include membrane receptors and enzymes.

Cannabinoids on the other hand, are now known to produce their psychotropic effects via high-affinity, specific interactions with relatively few trans-membrane receptors, the cannabinoid receptors.

There is a firmly established a role for the EC system in mediating the reinforcing properties of ethanol and the pathology of alcohol dependence. EC system is a molecular target for ethanol and how the function of this neuro-modulatory system is altered following chronic ethanol ingestion.

Given the CNS depressant effects of ethanol, the ability of acute ethanol administration to activate release of ECs seems, at first, to be contradictory as synthesis and release of ECs are highly dependent on excitability-induced elevations intracellular calcium ion concentrations.

However, brief incubation with ethanol is known to increase intracellular Ca2+ in forebrain synaptosomes and in hippocampal neurons via release from intracellular stores.

Most studies indicate that chronic ethanol treatment in rodents, regardless of whether ethanol administration is voluntary or not, causes decreased CB1 protein expression and G protein coupling. CB1 binding in Swiss–Webster mice subjected to 72 h of involuntary ethanol vapor exposure is decreased in the striatum indicating down-regulation of CB1 in this brain region (Vinod et al., 2006)

Some studies suggest that the effect of withdrawal on CB1 coupling, binding, and transcript levels is similar to what is observed for brain endocannabinoids with ethanol-induced effects on CB1 returning to normal following 24 h of ethanol deprivation.

Behavioral evidence

It has been proven the effect of cannabinoid agonists, antagonists, FAAH inhibitors or anandamide transporter inhibitors could alter the ethanol behavior in rodents.

Firstly, cannabinoid agonists WIN 55,212-2 and CP 55,940 cause an increase in ethanol consumption and preference. These agonists also increased the chances of relapse during alcohol deprivation, when tested in rats. Whereas the effect of cannabinoid antagonists rimonabant and surinabant reduced the alcohol consumption and related behaviors.

To understand the withdrawal and relapse in animals, two experiments were carried out. Firstly, the alcohol deprivation effect states the increased voluntary ethanol intake after a period of alcohol deprivation. What's interesting is how the cannabinoid antagonist rimonabant completely inhibited the alcohol deprivation effect in alcohol preferring rats. Secondly, animals were stimulated by a certain stimulus which was related to alcohol availability previously, after the stimulus the animals showcased ethanol seeking behavior. In the same way, cannabinoid antagonist rimonabant has reduced such behaviors.

Another cannabinoid antagonist surinabant was also tested, it exerts similar effect as rimonabant. Surinabant reduced alcohol intake and preference in alcohol preferring-rats and non-preferring rats that had been chronically alcoholised. It also reduced the likelihood of ethanol drinking in alcohol-preferring rats and decreased the alcohol deprivation effect.

In mice lacking CB1 receptors evidently reduced voluntary alcohol consumption occurs in a two bottle choice paradigm*. The ethanol withdrawal symptoms were totally absent. Knockout mice* with fatty acid amide hydrolase (FAAH) showed higher endocannabinoids level, increased preference and intake of ethanol. These mice also showed lower sensitivity to acute effects of ethanol and reduced ethanol withdrawal symptoms. However, no real difference in ethanol sensitivity or withdrawal were seen.

This section confirmed the stimulation of the endocannabinoid system stimulates the ethanol consumption, and the blockage of the system reduces ethanol consumption.

There are two hallmark behavioural features of addiction: an incredible overriding compulsion to seek and use and an inability to control or inhibit these actions even though they will likely result in a negative outcome .Studies have shown these two features of addiction emerge from aberrant learning processes and plasticity in the striatum and frontal cortices.

Ethanol and cannabis may serve as a substitute for one another when treating such behaviours. Ethanol and cannabis both have depressant effects on central nervous system function, inhibiting the aberrant processes.

In laboratory rodents, administration of either ethanol or D9- tetrahydrocannabinol produces hypolocomotion, hypothermia, and ataxia.

Ethanol's low affinity is evidenced by the fact that it must be consumed in quantities sufficient to produce blood concentrations in the millimolar range before substantial behavioral effects can be observed. In contrast, THC produces the majority of its effects through high-affinity interactions with a small number of molecular targets. Almost all of the centrally mediated, behavioural effects of THC intoxication result from activation of the CB1 receptor.

There are several behavioural ramifications of an altered EC signaling in the context of circuitry associated with addictive processes.

In a study that altered EC signalling, the authors reported cannabis significantly increased pulse, time estimation, and low-voltage, high-frequency EEG activity. Ethanol, on the other hand, was observed to only decrease subjective time estimation. It is important note that the authors reported that participants who claimed a history of heavy marijuana use were less intoxicated by ethanol and showed fewer ethanol-induced neuropsychological impairments than were found in previous studies.

In addition to their subjective and physiological effects, cannabis and ethanol are also known to affect cognitive function , such as in a study , ethanol and marijuana interact synergistically to impair cognitive and psychomotor functions when coadministered.

With regard to the cognitive effects of chronic ethanol and cannabis exposure, one study demonstrated significantly impaired learning in a Hebb-Williams maze and a punished moving belt test for rats that were chronically treated with either 6 g/kg ethanol or 10 mg/kg THC for six months and allowed to recover for one month be training . These studies suggest that although ethanol and cannabis have distinct subjective effects, these two drugs produce similar deficits in cognitive function after both acute and chronic administration.

Genetic evidence

Alcohol dependence is a heterogeneous disorder with multiple genetic backgrounds. Studies have shown as high as over 50% heritability. A single nucleotide polymorphism of the CB1 receptor (rs1049353) was associated with severe alcohol withdrawal syndrome. However, another study working with the same particular variation failed to prove the relation between the polymorphism and the phenotypic performance.

A nine-allele microsatellite polymorphism of the CB1 receptor containing repeats of AAT nucleotide sequence has been associated with childhood attention deficit/hyperactivity disorder in alcoholic patients. But again, another similar experiment was performed but the result did not show correlations with alcohol dependence but with other drugs. Even more studies were carried out, but they all failed to prove the association between the genome and alcohol dependence.

Three studies have provided positive association of CB1 receptor variants with alcohol dependence. The probability of alcohol dependence was strongly associated with rs6454674

single nucleotide polymorphism. Another study showed how the C allele of rs2023239 CB1 receptor single nucleotide polymorphism was associated with greater CB1 receptor concentration in the prefrontal cortex of alcoholic patients.

Single nucleotide polymorphism of the FAAH gene, the rs324420 is associated with drug and alcohol use. Gene on the CB2 receptor polymorphism rs2501432 is related to alcohol dependence.

Many studies have shown genetic evidence in the involvement of the endocannabinoid system in alcohol dependence. However, with the same experiments, not always the same positive results happened. So , it is evident more studies and experiments should be done in future to further specify and reassure the accuracy of the information.

Preclinical rodent models have been used to show that genetic and pharmacological inhibition of EC signalling can profoundly reduce voluntary ethanol consumption, reward for ethanol, as well as reinstatement and relapse of ethanol-motivated behaviours

Using the two bottle choice assay, CB1 knock-out (KO) mice have been shown to exhibit decreased voluntary drinking of ethanol. Consumption of 10% ethanol was decreased in CB1 KO mice given six or eight hours of limited access to ethanol per day .

Under continuous access conditions, ethanol consumption was decreased for multiple ethanol concentrations in both male and female CB1 KO mice compared to wild-type controls. However, the inhibitory effect of CB1 deletion on ethanol intake was more profound in female mice, which generally show higher overall basal ethanol consumption compared to their male counterparts .Also, age-related decreases in ethanol drinking were shown to be absent in CB1 KO mice, suggesting that the decrease in ethanol consumption that occurs during aging is mediated by endocannabinoid signaling .

In a separate study mice lacking CB1 exhibit marked differences in ethanol sensitivity. Mice lacking CB1 on either a C57Bl6 or DBA/2J genetic background display a longer duration of loss of righting reflex following systemic injection of either 2 or 4 g/kg ethanol

Alcohol intake and preference

Fatty acid amide hydrolase (FAAH) is a primary catabolic enzyme for anandamide. The inhibition or genetic deletion of FAAH increases anandamide level and augments alcohol consumption and preference. A single-nucleotide polymorphism (SNP; C385A, rs324420) in the FAAH gene makes FFAH more vulnerable to degradation. The elevated level of anandamide activity caused by the FAAH SNP is attributed to increased alcohol consumption in studies. On the other hand, CB1 receptor antagonist decreases alcohol intake and reinforcement. Thus, the endocannabinoid system plays an important role in alcohol intake: alcohol reinforcement and consumption (Zhou et al. 2016).

To investigate the specificity of the FAAH influence on alcohol intake, Yan Zhou et. al. conducted an experiment using $FAAH^{A/A}$ (C385A SNP) and $FAAH^{C/C}$ (wild-type) mice. They examined genetic differences in alcohol, sucrose, and saccharin preference in FAAH knock-in mice. After the 4-day exposure of 15 % alcohol (ethanol), the $FAAH^{A/A}$ mice consumed more alcohol than the $FAAH^{C/C}$ ones, showing a significant effect of genotype. On the other hand, as to sucrose preference, no genotypic difference was observed between $FAAH^{A/A}$ and $FAAH^{C/C}$ mice over the 4-day exposure under the same condition of the alcohol part. Same as sucrose, there was no genotype difference between them in saccharin preference. This leads to the conclusion that increased endocannabinoid signaling in $FAAH^{A/A}$ causes an increased level of alcohol intake and preference. The alcohol vulnerability derived from FAAH SNP C358A is confirmed (Zhou et al. 2016).

Alcohol tolerance and dependence

The endocannabinoid system also is related to alcohol tolerance and dependence. Basavarajappa (2019) explains these two terms,

- Tolerance: a lack of response to the repeated use of alcohol and leads to the need for higher volumes to experience the familiar effects.
- Dependence: a physical condition in which the body has adapted to the continued presence of alcohol and drives craving.

The cessation of chronic alcohol intake causes increased consumption of alcohol to avoid withdrawal effects.

Alcohol withdrawal reduces the density of CB1 receptors, and Chronic alcohol exposure leads to a decreased level of CB1 receptors. When cells undergo chronic alcohol exposure, both AEA and 2-AG contents are increased.

Alcohol self-administration and relapse

The pharmacological control on CB1 receptors displayed the different effects on alcohol intake. The inhibition of CB1 receptors lessens the rewarding properties of alcohol, and the activation brings about the opposite result.

The self-administration of alcohol shows the increase in the extracellular 2-AG level in the nucleus accumbens. This is linked with the amount of consumed alcohol (Caille et al. 2007). After CB1 receptors become activated, 2-AG undergoes hydrolysis in neurons by monoacylglycerol lipase (MAGL) (Lu & Anderson, 2017). MAGL inhibitors can increase anxiety-like behavior and alcohol intake, and thereby the pharmacological blockade of MAGL boosts alcohol consumption and preference as well (Serrano et al., 2018).

The goal of treatment of alcohol disorders is to prevent relapse. Since the endocannabinoid system plays a key role in various aspects of behaviors related to alcohol, it should be related to alcohol relapse. With the fact that CB1 receptor antagonists, such as SR, reduce alcohol intake, the inhibition of CB1 receptors affects alcohol self-administration. Also, the combined administration of SR with either an adenosine A2A or mGlu5 receptor antagonist was found to prevent relapse-like alcohol intake (Adams et al., 2010). On the other hand, the inability of SR exposure to affect foot-shock-elicited relapse suggests that CB1 receptors have no significant relation to stress-induced relapse (Economidou et al., 2007). Still, CB1 receptors play an essential role in alcohol relapse.

Susceptibility to alcohol abuse

As mentioned earlier, the FAAH C385A SNP enhances alcohol preference. In addition to it, the cannabinoid CB1 receptor gene (CNR1 allele) is found to be a key indicator of susceptibility to alcohol disorders (Marcos et al., 2012). Thus, endocannabinoid-related genes may contribute to higher risks of alcohol abuse.

CB2 receptor

The existence of CB2 receptors in the brain is controversial. However, CB2 receptors also take part in alcohol abuse behavior. The activation of CB2 receptors reduces alcohol intake. In other words, the lack of CB2 receptors leads to increased alcohol intake (Pradier et al., 2015). Also, the genetic ablation of the Cnr2 gene enhances the preference for and vulnerability to alcohol consumption (Navarrete et al., 2018).

Treatment of alcohol disorders

As previously discussed, the endocannabinoid system is greatly associated with alcohol dependence. Moreover, the use of antagonist rimonabant has shown its effectiveness in decreasing the ethanol and drug intake.

The first clinical testing happened throughout a 12 week period, placebo controlled study with the administration of 20mg of rimonabant per day in the prevention of alcohol relapse. 41.5% vs 47.7% was the average relapse rate, 27.7% vs 35.6% was the relapse rate to heavy drinking. The difference is relatively small, thus the authors suggested the possibility of the lack of improvement in rimonabant, firstly, the high beneficial response rate in the placebo group, secondly, by the short duration of the treatment.

The second clinical trial went through a course of 3 weeks, using a placebo controlled study with the effect of rimonabant 20mg per day on alcohol consumption in non-treatment seeking heavy alcohol drinkers. After a week, rimonabant did not alter alcohol consumption significantly. Finally, rimonabant did not change alcohol self-administration.

In the endocannabinoid system, CBD acts as a non-competitive antagonist of CB1 receptors, with negative allosteric modulation and a low affinity for CB1 receptors (Chye, Christensen, Solowij, & Yucel, 2019). In an experiment, the administration of CBD reduces alcohol intake in C57BL/6J mice. Hence, CBD is expected to be a key to the treatment of alcohol use disorders.

Conclusion

From the evidence displayed in the essay above, endocannabinoids show a clear role within excessive alcohol use, from a biochemical cross-tolerance to behavioural inhibiting actions. While there are a few genetic predispositions, the use of cannabinoids as a treatment for alcohol abuse , while still requiring further study and large scale trials, is a possibility in the near future.

Definitions

Anandamide: a naturally occurring endogenous cannabinoid neurotransmitter found in the brains of mammals

Alcohol deprivation effect: is a temporary increase in the ratio of ethanol/total fluid intake and the voluntary intake of ethanol solutions over baseline drinking conditions when ethanol access is reinstated after a period of alcohol deprivation.

Cannabinoid 1 (CB1) receptor: CB1 is a G-protein coupled receptor (GPCR) and is the main receptor for the endogenous cannabinoid (endocannabinoid; EC) system in the brain

D9-tetrahydrocannabinol (THC): the main psychoactive compound in cannabis

Ecs: a class of lipid-derived neuromodulators that serve as retrograde transmitters in central synapses

Knockout mice: A knockout mouse is a laboratory mouse in which researchers have inactivated, or "knocked out," an existing gene by replacing it or disrupting it with an artificial piece of DNA.

Two bottle choice paradigm: animals are presented with access to two bottles; typically, one contains an ethanol solution and the other contains a non-ethanol beverage (usually water)

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