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Research report

Functional interaction and cross-tolerance between ethanol and Δ^9-THC: Possible modulation by mouse cerebellar adenosinergic A_1/GABAergic-A receptors

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HIGHLIGHTS

- Chronic THC results in robust tolerance to THC and cross-tolerance to ethanol.
- Repeated ethanol results in robust tolerance to ethanol and cross-tolerance to THC.
- Chronic ethanol/THC leads to marked cross-tolerance to $GABA_A$/adenosine A_1 agonists.
- The cross-tolerance observed between ethanol/THC and muscimol/CHA was heterologous.

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ABSTRACT

We have previously shown a functional motor interaction between ethanol and Δ^9-tetrahydrocannabinol (Δ^9-THC) that involved cerebellar adenosinergic A_1 and GABAergic A receptor modulation. We now report the development of cross-tolerance between intracerebellar Δ^9-THC and intraperitoneal ethanol using ataxia as the test response in male CD-1 mice. The drugs [Δ^9-THC (20 μg), N^6-cyclohexyladenosine, CHA (12 ng), muscimol (20 ng)] used in the study were directly microinfused stereotaxically via guide cannulas into the cerebellum except ethanol. Δ^9-THC, infused once daily for 5 days followed 16 h after the last infusion by acute ethanol (2 g/kg) and Rotorod evaluation, virtually abolished ethanol ataxia indicating development of cross-tolerance. The cross-tolerance was also observed when the order of ethanol and Δ^9-THC treatment was reversed, i.e., ethanol injected once daily for 5 days followed 16 h after the last ethanol injection by Δ^9-THC infusion. The cross-tolerance appeared within 24–48 h, lasted over 72 h and was maximal in 5-day ethanol/Δ^9-THC-treated animals. Finally, tolerance in chronic ethanol/Δ^9-THC/-treated animals developed not only to ethanol/Δ^9-THC-induced ataxia, respectively, but also to the ataxia potentiating effect of CHA and muscimol, indicating modulation by cerebellar adenosinergic A_1 and $GABA_A$ receptors. A practical implication of these results could be that marijuana smokers may experience little or no negative effects such as ataxia following alcohol consumption. Clinically, such antagonism of ethanol-induced ataxia can be observed in marijuana users thereby encouraging more alcohol consumption and thus may represent a risk factor for the development of alcoholism in this segment of population.

1. Introduction

There is widespread abuse of both cannabinoids and ethanol with the current association of both substances in humans and their potential link to motor vehicle accidents [1]. Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the primary active psychoactive ingredient of the cannabis plants. The recreational use of both psychoactive drugs is primarily via smoking either in the form of cigarette or through a pipe, and drinking as alcoholic beverages, respectively. In spite of the current well known high frequency of consumption of both psychoactive substances in humans [2], the effects of

Abbreviations: ICB, intracerebellar; ANOVA, analysis of variance; S.E.M., standard error of mean; Δ^9-THC, Δ^9-tetrahydrocannabinol; GABA, γ-amino-butyric acid; mM, millimolar; nL, nanoliter; ng, nanogram; PKA, protein kinase A; cAMP, cyclic adenosine mono phosphate; cpt-cAMP, 8-(4-chlorophenylthio)-cAMP; CREB, cAMP response element-binding protein; CHA, N^6-cyclohexyladenosine; DMSO, dimethylsulfoxide; aCSF, artificial cerebrospinal fluid; AP, anterior–posterior; ML, medio-lateral; DV, dorso-ventral; MRI, magnetic resonance imaging; PET, positron emission tomography.

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co-administration of Δ^9-THC and alcohol and possible functional interactions are not well studied. Therefore, the obvious importance of understanding the mechanism of one of the important central nervous system effects of cannabinoids, such as ataxia, and the functional consequence of co-administration of both alcohol and Δ^9-THC, cannot be overemphasized. In addition, the study may further our knowledge about the role and functions of endocannabinoids.

We have previously reported that the accentuation of intracerebellar (ICB) Δ^9-THC-induced ataxia following systemic ethanol administration in mice suggests behavioral interaction between the two psychoactive substances in an adenosine A_1 receptor sensitive manner [3]. The combined effect of ICB Δ^9-THC and systemic alcohol administration, however, was found to be considerably worse on ataxia [3], and memory impairment [4] in mice and rats, respectively. Cross-tolerance between alcohol and Δ^9-THC was reported in earlier studies [5,6], but the results were contradictory due to variability in experimental conditions. It has also been observed that the effect of alcohol is attenuated in humans using marijuana suggesting possible cross-tolerance between the two substances [7,8].

Many reports implicate endocannabinoids in the modulation of alcohol-induced addiction [9] and behaviors [10], which is a further evidence of functional interactions between the two substances. For example, CB_1 agonists stimulate alcohol intake and craving [11], and alcohol alters brain endocannabinoids system [12,13]. Endocannabinoids within central nervous system tonically regulate inhibitory transmission and depress the effect of ethanol in various brain regions [14], due to CB_1 receptor activation by endogenous anandamide. Similarly, high doses of a CB_1 receptor antagonist SR141716A, has been associated with hyperactivity in rats [15], indicating a possible tonic activity of CB_1 receptors within the central nervous system.

The localization of CB_1 receptors to the molecular layer of the cerebellar cortex was the basis to target cerebellar cortex for the direct microinjection of Δ^9-THC and other drugs in the present investigation. Actions of the cannabinoids at the level of glutamate release from pre-synaptic terminals and at the level of GABA release from basket and/or stellate cells most likely account for observed motor effects [16]. However, presence of low density CB_1 receptors on the deep cerebellar nuclei has also been reported which may lend support to the concept of multiple sites of cannabinoid action within the cerebellum [17]. Pre-synaptic terminals of parallel fibers contain several G_i-protein-coupled receptors (such as $GABA_B$, CB_1, adenosine A_1 and κ-opioids) that share the same adenylate cyclase effector system for inhibition of cAMP formation [18,19]. Alternatively, the interaction between cannabinoid receptors and other $G_{i/o}$-linked receptors may occur at the level of inhibitory stellate and/or basket cells where co-localization of $G_{i/o}$-coupled receptors also exists.

Chronic systemic administration of Δ^9-THC consistently produces a rapid tolerance [20]. However, in spite of dramatically different experimental conditions, we have also observed in the present investigation development of a rapid and robust tolerance within 3–5 days following direct microinfusion of Δ^9-THC into the cerebellar cortex. To the best of our knowledge, no study involving co-administration of systemic ethanol and direct ICB microinfusion of Δ^9-THC on ataxia has been reported. We report development of virtually total tolerance to acute systemic ethanol, following chronic ICB microinfusion of Δ^9-THC (i.e., cross-tolerance developed between ICB Δ^9-THC and systemic ethanol). However, unlike previous literature report [21], the cross-tolerance between the two psychoactive substances was equally strong regardless if the chronic ICB microinfusion of Δ^9-THC was followed by systemic acute ethanol or vice versa. The adenosine A_1 $G_{i/o}$-coupled receptor is co-localized with the CB_1 $G_{i/o}$-coupled receptor, and most likely shares similar regulatory pathway with the CB_1 receptor. Such evidence already exists for $GABA_B$ and adenosine A_1 receptors [22]. Additionally, we have previously demonstrated cerebellar adenosinergic A_1 and GABAergic modulation of ethanol-induced ataxia [23,24]. Interestingly, Δ^9-THC-induced ataxia is also modulated by cerebellar adenosine A_1 receptor system [3,24]. Consequently, we hypothesize that the development of cross-tolerance between ethanol and Δ^9-THC may also involve tolerance to both adenosine A_1 and $GABA_A$ receptor agonist drugs. We tested the hypothesis by comparing the motor-impairing effects of co-administered doses of Δ^9-THC and ethanol by Rotorod. In addition, we have also conducted detailed assessment of the development of cross-tolerance between ethanol and Δ^9-THC, again using the Rotorod test. Finally, the effect of $GABA_A$ and adenosine A_1 agonist drugs on the development of tolerance and cross-tolerance between ethanol and Δ^9-THC was investigated. We report observation of robust heterologous cross-tolerance between chronic ethanol and acute ICB microinfusion of Δ^9-THC + CHA/muscimol as well as chronic Δ^9-THC (ICB) and acute systemic alcohol + CHA/muscimol (ICB).

2. Materials and methods

2.1. Experimental subjects

The present investigation involved the use of male CD-1 mice (Charles River, Raleigh, NC) weighing 22–26 g at the time of surgery and 5–6 weeks of age. Immediately after arrival, animals were subjected to 5-day mandatory rest and recovery. They were housed in groups of eight in Plexiglas cages in temperature- and humidity-controlled animal housing, lights from 8:00 AM to 8:00 PM. However, following stereotaxic implantation of the stainless steel guide cannulas in a survival surgery, the animals were transferred to individual plastic cages with stainless steel wire lid. They were provided commercial pellet food and water ad libitum except during the Rotorod behavioral test. The East Carolina University Institutional Animal Care and Use Committee approved the use of animals for the procedures in the study including aseptic stereotaxic surgery under animal use protocol number AUP W125e. Laboratory facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and principles of laboratory animal care were consistent with the Declaration of Helsinki and Guide for the Care and Use of Laboratory Animals as adopted by National Institutes of Health.

2.2. Drugs

Drug solutions were either prepared on the day of behavioral experiments or prepared a day earlier and kept in the deep freezer at −70 °C. Chloral hydrate was prepared in sterile normal saline and injected at a volume of 0.1 ml/10 g body weight. The sources for the drugs used in the study were as follow: chloral hydrate, muscimol (Sigma–Aldrich, St. Louis, MO, USA); N^6-cyclohexyladenosine (CHA) (Research Biochemicals, Natick, MA, USA). The CB_1 receptor agonist Δ^9-THC, provided by the Department of Human Health Services (DHHS) from the National Institute of Drug Abuse (NIDA), Research Triangle Institute (Research Triangle Park, NC), was dissolved in 100% dimethylsulfoxide (DMSO). Unless otherwise stated, all other drugs were dissolved in artificial cerebrospinal fluid (aCSF) containing (mM): NaCl, 127.65; KCl, 2.55; $CaCl_2$, 0.05; $MgCl_2$, 0.94; $Na_2S_2O_5$, 0.05; at pH 7.4. The CHA was dissolved in aCSF with the aid of minimal volume of DMSO. A remarkable consistency in the ataxia-producing effect of ethanol was achieved and maintained by using the same vendors for mice and ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY, USA). The solution of ethanol (10%,