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SHORT-TERM EXPOSURE TO ALCOHOL IN RATS AFFECTS BRAIN LEVELS OF ANANDAMIDE, OTHER *N*-ACYLETHANOLAMINES AND 2-ARACHIDONOYL-GLYCEROL

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Abstract

Chronic alcohol exposure leads to significant changes in the levels of endocannabinoids and their receptors in the brains of humans and laboratory animals, as well as in cultured neuronal cells. However, little is known about the effects of short-term periods of alcohol exposure. In the present study, we examined the changes in endocannabinoid levels (anandamide and 2-arachidonoylglycerol), as well as four additional *N*-acylethanolamines, in four brain regions of rats exposed to alcohol through the liquid diet for a period of 24 hours. The levels of *N*-acylethanolamines were diminished 24 hours after the onset of alcohol exposure. This was particularly evident for anandamide in the hypothalamus, amygdala and caudate-putamen, for *N*-palmitoylethanolamine in the caudate-putamen, for *N*-oleoylethanolamine in the hypothalamus, caudate-putamen and prefrontal cortex, and for *N*-stearoylethanolamine in the amygdala. The only exception was *N*-linoleoylethanolamine for which the levels increased in the amygdala after the exposure to alcohol. The levels of the other major endocannabinoid, 2-arachidonoylglycerol, were also reduced with marked effects in the prefrontal cortex. These results support the notion that short-term alcohol exposure reduces endocannabinoid levels in the brain accompanied by a reduction in several related *N*-acylethanolamines.

Keywords

Alcohol; endocannabinoids; anandamide; 2-arachidonoylglycerol; *N*-acylethanolamines; brain

INTRODUCTION

The cannabinoid system has been implicated in several neuropsychiatric disorders [17,37]. This idea is based on two observations: (i) that its activity is altered during the progression of

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these disorders, and (ii) that its pharmacological management alleviates specific symptoms (see [17,37] for review). Drug addiction is one of the neuropsychiatric disorders where the cannabinoid system has been implicated [28]. There is evidence for dependence to cannabis [22], whereas the cannabinoid system is involved in the dependence to opioids [38], nicotine [10], and other drugs (see [31] for review). A clinically promising case is alcohol addiction, this owing to the relevant and numerous recent articles citing preclinical evidence for its role (see [11,25,26] for review). These studies demonstrated that chronic alcohol exposure elicits a neuroadaptative response, which involves the cannabinoid signaling system in the brain among others [11,25,26]. This may explain the influence of the cannabinoid system in the expression of several behaviors associated with alcohol addiction such as preference, intake, vulnerability, craving, withdrawal and relapse [11,25,26]. The adaptative changes caused by alcohol in the cannabinoid system appear to be similar to those that alcohol provokes in most brain neurotransmitters, especially GABA and glutamate but also monoamines and several neuropeptides (see [9,12] for a recent review). These changes occur as an attempt of the brain to counteract the action exerted by continuously present alcohol [14]. In the case of the cannabinoid system, the effects of alcohol have been reported in both cultured cells [2,3] and laboratory animals [4,20,30] where alcohol affects both endocannabinoid ligands [2–4,21,39] and their receptors [1,4,20,30,39]. Most of these studies reported an increase of the two major endocannabinoid ligands, anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), after prolonged alcohol exposure [2,3,5,19,39]. By contrast, there are some controversial results regarding the changes in receptors for these endocannabinoids after alcohol exposure, since some studies described downregulation/desensitization responses of CB_1 receptors [1,4,30,39], whereas other studies reported no changes [20].

An important aspect of the above-referenced studies is that all were conducted after prolonged exposure to alcohol, this in an attempt to develop *in vivo* and *in vitro* models that reproduce the pattern of long-term consumption characteristic of alcoholic humans. However, there is little information on the short-term effects of alcohol on cannabinoid signaling. A recent study by Ferrer et al. [15] reported that an acute injection of alcohol reduced AEA levels in several brain regions and peripheral tissues. In the present study, we further explored the issue by analyzing the effects of a short-term (24 hours) exposure to alcohol on the levels of the two major endocannabinoids, AEA and 2-AG, in the rat brain. The analyses were done in brain areas related to motor (caudate-putamen), feeding (hypothalamus) and stress/emotion (amygdala and prefrontal cortex) behaviors, these being different from those analyzed (ventral striatum, cerebellum and hippocampus) in the study by Ferrer et al. [15]. We also examined the effect of short-term alcohol exposure on the levels of representative *N*-acylethanolamines (NAEs), because alcohol exposure frequently affects several enzymes (e.g., fatty acid amide hydrolase (FAAH) [7,33,39]) that are involved in the metabolism of AEA, 2-AG and other structurally-related compounds. These included *N*-oleoylethanolamine (OEA), *N*-palmitoylethanolamine (PEA), *N*-linoleoylethanolamine (LEA) and *N*-stearoylethanolamine (SEA). The cannabinoid-like pharmacological effects *in vivo* of these NAEs, contrary to those of AEA and 2-AG which bind to and activate cannabinoid CB_1 or CB_2 receptors, cannot be attributed to activation of any of the known cannabinoid receptor types (for review, see [24]), although some of them are active at the related TRPV1 receptor [29]. Rather, NAEs potentiate the action of both endocannabinoids at their receptors through an “entourage effect” (for review, see [16]), which appears to result from a NAE-dependent protection of endocannabinoids from their usual inactivation mechanisms [24].

MATERIALS AND METHODS

Animals, treatments and sampling

Male Sprague-Dawley rats were maintained on a 12:12h light-dark cycle (07:00 light on) under controlled conditions of temperature and humidity. Food was available *ad libitum*. Animals were used at adult age (500–550 g weight) for experimental purposes. All experiments follow the guidelines of the National Institutes of Health (USA) for animal care and experimentation. The experiments consisted of placing the animals in cages (n=4/cage) where they were allowed to drink alcohol (ethanol 8% v/v) in the liquid diet, according to the procedure described by Uzbay et al. [36], over a period of 24 hours. Control animals were allowed to drink only water. Details that confirm the utility of this method to develop experimental conditions for short- or long-term exposures to alcohol have been published previously [19,20]. Briefly, the consumption of liquid diet by animals during the period of 24 hours was 69.34 ± 2.34 ml/kg weight for the control group (n=4 animals) and 63.63 ± 1.14 ml/kg weight ($p<0.05$) for alcohol-exposed group (n=8 animals). This led to an alcohol exposure equivalent to 7.83 g/kg weight in the animals allowed to drink a solution with 8% ethanol. No evidence of behavioral effects or signs of intoxication were observed in these animals. 24 hours after the onset of alcohol exposure, animals were euthanized by decapitation. Their brains were dissected on an ice-cold glass dissection plate and the following regions were collected: hypothalamus, amygdala, caudate-putamen and prefrontal cortex. Dissected samples were weighed, frozen on dry ice, and stored in eppendorf tubes containing ice-cold methanol at −80°C until lipid extraction and analysis. The entire procedure took less than 10 min, which is significantly less than the amount of time required for postmortem generation of 2-AG and AEA [27].

Analysis of brain levels of endocannabinoids and related-NAEs

Lipid extraction—All samples corresponding to the same brain region were processed on the same day. A minimum of 20 volumes of methanol were added to each sample. [$_2H^8$]-Anandamide (10μM; Cayman Chemical, Ann Arbor, MI, USA) was also added to be used as an internal standard to track the recovery of the test compounds. Then, the samples were homogenized, and the homogenates were centrifuged for 20 min at 15,000 rpm. Supernatants were collected and transferred to polypropylene 15 ml or 50 ml centrifuge tubes. Water was added to each sample to create a water/methanol (75/25) solution. Analytes were extracted from the supernatant using C18 solid-phase extraction columns (500 mg; Varian, Harbor City, CA, USA). Each column was conditioned with 2.5 ml 100% methanol and 1.5 ml water, followed by loading of the water/supernatant solution. Columns were then washed with water (1.5 ml) followed by 85% methanol (1.5 ml). In the case of prefrontal cortex samples, which weighed significantly more than the other regions, the columns were additionally washed with 60% methanol (1.5 ml) between water and 85% methanol. Compounds were eluted with 1.5 ml methanol. Each eluted sample was vortexed at maximum speed for 1 min before mass spectrometric analysis. The entire procedure was carried out on ice, and all solvents were HPLC-/mass spectrometrygrade.

Analysis and quantification—Samples were analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Rapid separation was obtained using 10 μl injections of analyte (Agilent 1100 series autosampler, Wilmington, DE) onto a Zorbax eclipse XDB 2.1X 50-mm C18 reversed-phase column. Gradient elution (200 μl/min) was formed under pressure on a pair of Shimadzu 10AdVP pumps (Columbia, MD). Mass spectrometric analysis was performed with an Applied Biosystems/MDS Sciex (Foster City, CA) API 3000 triple quadrupole mass spectrometer using electrospray ionization. Levels of each compound were analyzed by multiple reaction monitoring (MRM) on the LC/MS/MS system. The molecular and fragment ions used were as follows: 2-AG, 379.3 →287.3; AEA, 348.3 →62.5; PEA, 300.3 →62.5; LEA, 324.3 →62.5; OEA, 326.3 → 62.5; and SEA, 328.3 →62.5.

Data analysis

Data were analyzed by the two-way (alcohol treatment × brain region) analysis of variance followed by the Student-Newman-Keuls test, with GraphPad Prism 4 (San Diego, CA) software. P<0.05 was considered statistically significant.

RESULTS

The levels of AEA, 2-AG and related NAEs measured in control animals varied according to region but were normally within the range of concentrations described in previous studies [6, 8,24]. Thus, the highest levels of AEA were found in the amygdala [$F(3,40)=36.62$, $p<0.0001$], for 2-AG [$F(3,40)=116.81$, $p<0.0001$] and SEA [$F(3,40)=38.29$, $p<0.0001$] in the hypothalamus, and for LEA [$F(3,40)=2.07$, $p=0.119$], PEA [$F(3,40)=135.51$, $p<0.0001$] and OEA [$F(3,40)=32.14$, $p<0.0001$] in the caudate-putamen (see Table 1). By contrast, the lowest values were found in the prefrontal cortex for AEA, 2-AG and SEA, and in the amygdala for LEA, PEA and OEA (see Table 1).

Short-term exposure to alcohol produced statistically significant effects in all compounds analyzed as revealed the statistics for the variable “alcohol treatment” [AEA: $F(1,40)=24.4$, $p<0.0001$; 2-AG: $F(1,40)=6.59$, $p<0.05$; PEA: $F(1,40)=13.73$, $p<0.001$; OEA: $F(1,40)=24.25$, $p<0.0001$; SEA: $F(1,40)=14.21$, $p<0.0005$]. The only exception is LEA for which only the 2-way interaction (brain region × alcohol treatment) reached statistically significance [$F(3,40)=3.54$, $p<0.05$] in concordance with its particular pattern of region-by-region changes (see below). The post-hoc analysis revealed that alcohol exposure produced statistically significant reductions in AEA and OEA levels in the hypothalamus, whereas trends towards a decrease were observed for 2-AG ($p=0.084$), PEA ($p=0.092$) and SEA ($p=0.081$), and no changes in LEA levels were found (Table 1). Relatively similar patterns were evident in the caudate-putamen and the prefrontal cortex. In the caudate-putamen, the reductions produced by alcohol exposure reached statistical significance in the case of AEA, OEA and PEA levels, but remained only as trends in the case of SEA ($p=0.087$) and, to a lesser extent, also LEA ($p=0.132$) (Table 1). 2-AG levels did not change with the treatment (Table 1). In the prefrontal cortex, 2-AG and OEA levels decreased in alcohol-exposed animals, whereas no changes were observed for the levels of PEA and SEA, and some trends towards a decrease were evident in the case of AEA ($p=0.132$) and, in particular, LEA ($p=0.093$) (Table 1).

In contrast with the above regions, the amygdala exhibited a different pattern. It was the only region where the exposure to alcohol caused an increase, and this was observed only in the case of LEA levels (Table 1). The remaining compounds responded to alcohol exposure as they usually did in the other regions, with statistically significant reductions in the case of AEA and SEA levels, a trend towards a decrease in the case of PEA levels ($p=0.055$), and no changes for 2-AG and OEA (Table 1).

DISCUSSION

The present results revealed that a short-term alcohol exposure in rats produced multiple changes (most of them reductions) in the levels of endocannabinoids and related NAEs in brain areas related to emotion, feeding and movement. These results confirm that short-term exposure to alcohol initiates multiple variations in the levels of these endogenous compounds, in concordance with those reported using prolonged periods of alcohol exposure [2–5,19,21, 30,39]. In addition, they show that these changes are specific to particular brain areas, indicating that each compound fluctuates according to a pattern specific to each brain region, although some common responses are also evident. Furthermore, these results suggest that the alcohol-induced changes affect not only the major endocannabinoid ligands, AEA and 2-AG, but also to several *N*-acylethanolamines, such as LEA, OEA, SEA and PEA. This is significant because

these compounds appear to influence the action of endocannabinoids at their receptors [16, 24].

The data revealed that AEA levels were significantly lower in the hypothalamus, caudate-putamen and amygdala of animals exposed to alcohol, and the same tendency was apparent in the prefrontal cortex. These results agree with *in vitro* results reported by Basavarajappa et al. [2], who described an initial decrease of AEA levels after the exposure of SK-N-SH cells to ethanol for 24 h. Similarly, our results agree with the results reported recently by Ferrer et al. [15], who described a reduction in AEA levels in areas different from those used in this study (cerebellum, hippocampus and ventral striatum). They also described a reduction in PEA levels, but they did not measure the levels of other NAEs or the other major endocannabinoid 2-AG. They used i.p. injections of alcohol and tested the levels of AEA and PEA at exposure times even shorter (45–90 min) than those used in our study.

We also found a marked decrease in 2-AG levels in the prefrontal cortex of alcohol-exposed animals. This contrasts with the increase in 2-AG described by Basavarajappa et al. [3] in cerebellar granule neurons exposed to alcohol for 24 to 72 h, thus strengthening the conclusion that the effects of short-term exposure to alcohol vary from one brain region to another. Therefore, it appears that different neurons from diverse brain regions respond to alcohol in a different manner or with a different time-course.

As mentioned above, PEA levels were also decreased after an acute alcohol exposure in the recent study by Ferrer et al. [15]. Our results are in agreement with this observation, since we found a general decrease after acute alcohol exposure in the levels of both unsaturated and saturated fatty acid ethanolamides in most of the brain areas examined. In particular, PEA levels were significantly decreased in the caudate-putamen, while OEA levels were significantly reduced in the caudateputamen, prefrontal cortex and hypothalamus and SEA levels in the amygdala. The only exception was LEA, the levels of which were markedly increased in the amygdala but not other areas of alcohol-treated animals. The chemical family of different NAEs share biochemical mechanisms for synthesis and metabolism with AEA and, to a lesser extent, 2-AG [34]. It is therefore likely that the mechanisms by which alcohol leads to a reduction in AEA and NAE levels may be similar. An attractive option is FAAH, the enzyme that degrades NAEs including AEA. However, FAAH activity was not altered by acute administration of alcohol in the study by Ferrer et al. [15], although FAAH is a major target for alcohol in the studies using chronic alcohol exposure [7], even in alcoholic humans [33]. Similar to the case of FAAH, Ferrer et al. [15] were also unable to find any effect of alcohol on enzymes related to the synthesis of AEA and other NAEs, such as *N*-acyltransferase and phospholipase D, which otherwise might have been candidate as mediators of the effects of alcohol found here. It is possible that the time-course used by Ferrer et al. [15] was too short to observe alteration in these enzymes. However, this hypothesis would require further confirmation.

Another aspect to consider is the possible involvement of different neurotransmitters, namely GABAergic or glutamatergic neurons, in the effects of alcohol on cannabinoid signalling [4, 11]. One of the best known properties of acute alcohol is the inhibition of excitatory neurotransmission. Although the molecular mechanisms remain to be elucidated, there is a broad consensus that both NMDA and non-NMDA (AMPA and kainate) glutamate receptors are inhibited by alcohol in several brain regions, thus altering the conductance of several ions including calcium (see [35] for review). Since enzymes participating in the synthesis of NAEs (phospholipase D and *N*-acyltransferase) require the presence of calcium [23,24] and alcohol reduces glutamate release and calcium entry in cells [35], one may hypothesize that the general reduction in the levels of both endocannabinoids and the other NAEs could be due to the action of alcohol on the glutamatergic system. This hypothesis would be in agreement with the results

of Ferrer et al. [15], where the decrease in AEA and PEA levels by acute alcohol was accompanied by decreased release of glutamate. This occurred in different areas despite the lack of changes in the enzymes involved in NAE synthesis. A similar mechanism may be operating here, although additional experiments would be required to confirm this hypothesis. Interestingly, this mechanism might lead to tolerance when the exposure to alcohol becomes regular, as this would result in an elevation of glutamate receptor-mediated signaling and increased production of endocannabinoids as reported by others (see [11,25,26] for review).

Taken together, our findings show that even short-term exposure to alcohol initiates multiple changes (mostly reductions) in the production of endocannabinoids and NAEs in specific brain regions implicated in stress/emotion, feeding, and motor-related processes, areas that are linked to the development of alcohol addiction. We propose that these short-term decreases in the endogenous compounds studied here may be related to the onset of neuroadaptative events associated with chronic consumption of alcohol, and some of the behavioral effects (i.e., irritability, attentional impairment, rewarding effects, motor incoordination, hypnotic effects) elicited by alcohol when consumed for a short period of time [13,18,32].

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Abbreviations

AEA	anandamide
2-AG	2-arachidonoylglycerol
FAAH	fatty acid amide hydrolase
LEA	*N*-linoleoylethanolamine
NAE	*N*-acylethanolamine
OEA	*N*-oleoylethanolamine
PEA	*N*-palmitoylethanolamine
SEA	*N*-stearoylethanolamine

REFERENCES

1. Basavarajappa BS, Cooper TB, Hungund BL. Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. Brain Res 1998;793:212–218. [PubMed: 9630633]
2. Basavarajappa BS, Hungund BL. Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor N-arachidonoylphosphatidylethanolamine in SK-N-SH cells. J. Neurochem 1999;72:522–528. [PubMed: 9930723]
3. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specifc neuromodulators in cerebellar granule neurons. Biochim. Biophys. Acta 2000;1535:78–86. [PubMed: 11113634]
4. Basavarajappa BS, Hungund BL. Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. Prostaglandins Leukot. Essent. Fatty Acids 2002;66:287–299.
5. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. Eur. J. Pharmacol 2003;466:73–83. [PubMed: 12679143]

6. Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, Fernández-Ruiz J, Di Marzo V. Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. Biochem. Biophys. Res. Commun 1999;256:377–380. [PubMed: 10079192]
7. Blednov YA, Cravatt BF, Boehm SL 2nd, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. Neuropsychopharmacology. 2007 in press.
8. Bradshaw HB, Rimmerman N, Krey JF, Walker JM. Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. Am. J. Physiol. Regul. Integr. Comp. Physiol 2006;291:R349–R358. [PubMed: 16556899]
9. Chastain G. Alcohol, neurotransmitter systems, and behavior. J. Gen. Psychol 2006;133:329–335. [PubMed: 17128954]
10. Cohen C, Kodas E, Griebel G. CB1 receptor antagonists for the treatment of nicotine addiction. Pharmacol. Biochem. Behav 2005;81:387–395. [PubMed: 15935455]
11. Colombo G, Serra S, Vacca G, Carai MA, Gessa GL. Endocannabinoid system and alcohol addiction: pharmacological studies. Pharmacol. Biochem. Behav 2005;81:369–380. [PubMed: 15939463]
12. Cowen MS, Lawrence AJ. Alcoholism and neuropeptides: an update. CNS Neurol. Disord. Drug Targets 2006;5:233–239. [PubMed: 16611096]
13. Dar MS, Mustafa SJ. Acute ethanol/cannabinoid-induced ataxia and its antagonism by oral/systemic/intracerebellar A1 adenosine receptor antisense in mice. Brain Res 2002;957:53–60. [PubMed: 12443980]
14. De Witte P, Pinto E, Ansseau M, Verbanck P. Alcohol and withdrawal: from animal research to clinical issues. Neurosci. Biobehav. Rev 2003;27:189–197. [PubMed: 12788332]
15. Ferrer B, Bermúdez-Silva FJ, Bilbao A, Alvarez-Jaimes L, Sánchez-Vera I, Giuffrida A, Serrano A, Baixeras E, Navarro M, Parsons LH, Piomelli D, Rodríguez de Fonseca F. Regulation of brain anandamide by acute administration of ethanol. Biochem. J. 2007 in press.
16. Fowler CJ. Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents. Non-psychoactive cannabinoids, 'entourage' compounds and inhibitors of N-acyl ethanolamine breakdown as therapeutic strategies to avoid pyschotropic effects. Brain Res. Rev 2003;41:26–43. [PubMed: 12505646]
17. Gambi F, De Berardis D, Sepede G, Quartesan R, Calcagni E, Salerno RM, Conti CM, Ferro FM. Cannabinoid receptors and their relationships with neuropsychiatric disorders. Int. J. Immunopathol. Pharmacol 2005;18:15–19. [PubMed: 15698507]
18. Giancola PR. Irritability, acute alcohol consumption and aggressive behavior in men and women. Drug Alcohol Depend 2002;68:263–274. [PubMed: 12393221]
19. González S, Cascio MG, Fernández-Ruiz J, Fezza F, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. Brain Res 2002;954:73–81. [PubMed: 12393235]
20. González S, Fernández-Ruiz J, Sparpaglione V, Parolaro D, Ramos JA. Chronic exposure to morphine, cocaine or ethanol in rats produced different effects in brain cannabinoid CB1 receptor binding and mRNA levels. Drug Alcohol Depend 2002;66:77–84. [PubMed: 11850139]
21. González S, Valenti M, de Miguel R, Fezza F, Fernández-Ruiz J, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in reward-related brain regions of alcohol-exposed rats, and their possible relevance to alcohol relapse. Br. J. Pharmacol 2004;143:455–464. [PubMed: 15371286]
22. González S, Cebeira M, Fernández-Ruiz J. Cannabinoid tolerance and dependence: a review of studies in laboratory animals. Pharmacol. Biochem. Behav 2005;81:300–318. [PubMed: 15919107]
23. Hansen HS, Lauritzen L, Strand AM, Vinggaard AM, Frandsen A, Schousboe A. Characterization of glutamate-induced formation of N-acylphosphatidylethanolamine and N-acylethanolamine in cultured neocortical neurons. J. Neurochem 1997;69:753–761. [PubMed: 9231736]
24. Hansen HS, Moesgaard B, Petersen G, Hansen HH. Putative neuroprotective actions of N-acyl-ethanolamines. Pharmacol. Ther 2002;95:119–126. [PubMed: 12182959]
25. Hungund BL, Basavarajappa BS. Are anandamide and cannabinoid receptors involved in ethanol tolerance? A review of the evidence. Alcohol Alcohol 2000;35:126–133. [PubMed: 10787386]

26. Hungund BL, Basavarajappa BS, Vadasz C, Kunos G, Rodriguez de Fonseca F, Colombo G, Serra S, Parsons L, Koob GF. Ethanol, endocannabinoids, and the cannabinoidergic signaling system. Alcohol Clin. Exp. Res 2002;26:565–574. [PubMed: 11981134]
27. Kempe K, Hsu FF, Bohrer A, Turk J. Isotope dilution mass spectrometric measurements indicate that arachidonylethanolamide, the proposed endogenous ligand of the cannabinoid receptor, accumulates in rat brain tissue post mortem but is contained at low levels in or is absent from fresh tissue". J. Biol. Chem 1996;271:17287–17295. [PubMed: 8663381]
28. Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 2006;29:225–232. [PubMed: 16483675]
29. Movahed P, Jonsson BA, Birnir B, Wingstrand JA, Jorgensen TD, Ermund A, Sterner O, Zygmunt PM, Hogestatt ED. Endogenous unsaturated C18 N-acylethanolamines are vanilloid receptor (TRPV1) agonists. J. Biol. Chem 2005;280:38496–38504. [PubMed: 16081411]
30. Ortiz S, Oliva JM, Perez-Rial S, Palomo T, Manzanares J. Chronic ethanol consumption regulates cannabinoid CB1 receptor gene expression in selected regions of rat brain. Alcohol Alcohol 2004;39:88–92. [PubMed: 14998822]
31. Parolaro D, Vigano D, Rubino T. Endocannabinoids and drug dependence. Curr. Drug Targets CNS Neurol. Disord 2005;4:643–655. [PubMed: 16375682]
32. Schreckenberger M, Amberg R, Scheurich A, Lochmann M, Tichy W, Klega A, Siessmeier T, Grunder G, Buchholz HG, Landvogt C, Stauss J, Mann K, Bartenstein P, Urban R. Acute alcohol effects on neuronal and attentional processing: striatal reward system and inhibitory sensory interactions under acute ethanol challenge. Neuropsychopharmacology 2004;29:1527–1537. [PubMed: 15085090]
33. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc. Natl. Acad. Sci. USA 2002;99:8394–8399. [PubMed: 12060782]
34. Sugiura T, Kishimoto S, Oka S, Gokoh M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog. Lipid Res 2006;45:405–446. [PubMed: 16678907]
35. Tsai G, Coyle JT. The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. Annu. Rev. Med 1998;49:173–184. [PubMed: 9509257]
36. Uzbay IT, Usanmaz SE, Akarsu ES. Effects of chronic ethanol administration on serotonin metabolism in various regions of the rat brain. Neurochem. Res 2000;25:257–262. [PubMed: 10786710]
37. Van der Stelt M, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. Eur. J. Pharmacol 2003;480:133–150. [PubMed: 14623357]
38. Vigano D, Rubino T, Parolaro D. Molecular and cellular basis of cannabinoid and opioid interactions. Pharmacol. Biochem. Behav 2005;81:360–368. [PubMed: 15927245]
39. Vinod KY, Yalamanchili R, Xie S, Cooper TB, Hungund BL. Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. Neurochem. Intern 2006;49:619–625.

Table 1

Endocannabinoids and *N*-acetylethanolamine levels in the hypothalamus, amygdala, caudate-putamen and prefrontal cortex of rats exposed to alcohol for 24 h and their corresponding controls

	HYPOTHALAMUS		**AMYGDALA**	
Compound levels (pmol/g tissue)	**Control**	**Alcohol**	**Control**	**Alcohol**
AEA	30.06 ± 3.46	20.32 ± 2.46 *	53.18 ± 5.13	32.83 ± 3.14 **
2-AG	3311.7 ± 258.4	2659.1 ± 202.0	1935.8 ± 196.6	1744.5 ± 93.0
LEA	5.77 ± 0.98	4.40 ± 0.57	3.36 ± 0.60	6.09 ± 0.70 *
PEA	290.6 ± 14.2	249.5 ± 13.7	132.9 ± 12.5	104.1 ± 7.1
OEA	341.4 ± 27.9	267.4 ± 15.3 *	204.5 ± 25.6	164.9 ± 16.4
SEA	79.9 ± 5.1	65.1 ± 4.7	52.1 ± 2.6	37.2 ± 3.2 *
	CAUDATE-PUTAMEN		PREFRONTAL CORTEX	
Compound levels pmol/g tissue)	Control	Alcohol	Control	Alcohol
AEA	25.6 ± 2.1	19.8 ± 1.3 *	16.7 ± 1.4	14.6 ± 0.6
2-AG	1022.6 ± 98.4	974.9 ± 56.7	558.7 ± 31.8	413.0 ± 15.4 ***
LEA	8.9 ± 3.1	5.2 ± 0.6	6.8 ± 1.2	5.1 ± 0.3
PEA	337.3 ± 10.9	293.8 ± 12.3 *	144.6 ± 2.8	134.9 ± 5.7
OEA	386.9 ± 22.3	293.1 ± 13.6 **	240.9 ± 16.1	196.8 ± 9.2 *
SEA	65.7 ± 4.4	58.4 ± 1.7	38.2 ± 3.1	36.7 ± 1.1

Data are means ± SEM of 8 animals in the alcohol group and 4 in the control group. Values were assessed by the two-way (brain region × alcohol treatment) analysis of variance followed by the Student-Newman-Keuls test (*p0.05, **p<0.01 and ***p<0.001 *versus* the control group).