CHRONIC ETHANOL CONSUMPTION REGULATES CANNABINOID CB₁ RECEPTOR GENE EXPRESSION IN SELECTED REGIONS OF RAT BRAIN

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Abstract — Aims: The aim of this study was to examine the effects of chronic ethanol consumption in cannabinoid CB₁ receptor gene expression in Wistar rats. Methods: Rats were exposed to a bottle containing a solution of ethanol (10% v/v) and saccharin (0.25% w/v) for 52 days. At the end of this period, rats were killed by decapitation and cannabinoid CB₁ receptor gene expression was measured by in situ hybridization histochemistry. Results: Our results indicated that chronic ethanol consumption reduced cannabinoid CB₁ receptor gene expression in caudate-putamen (CPu) (24%), ventromedial nucleus of the hypothalamus (VMN) (43%), CA1 (27%) and CA2 (22%) fields of hippocampus and increased dentate gyrus (DG) (30%). Conclusions: These results reveal for the first time that prolonged exposure to ethanol produces marked alterations in cannabinoid CB₁ receptor gene expression in selected regions of the rat brain, supporting an interaction between ethanol consumption and the endogenous cannabinoid receptor. Furthermore, these findings suggest that cannabinoid CB₁ receptor may be considered as a new pharmacological target for treating ethanol dependence.

INTRODUCTION

Genetic and environmental factors contribute to development of ethanol dependence. In the brain, ethanol dependence occurs after progressive disruption of homeostatic regulation of several neurotransmitters and hormones. Indeed, continued ethanol consumption induces pronounced alterations in a great variety of neurochemical elements (dopamine, endogenous opioids, GABA, serotonin, corticotropin releasing factor). These neurotransmitters and neuromodulators may be considered to underlie the molecular mechanisms of some anticonvulsant drugs (naltrexone and acamprosate) designed to reduce consumption of ethanol. Therefore, the study of new modulators of neuronal activity that are modified after prolonged exposure to ethanol contributes to the identification of neurobiological mechanisms involved in the development of dependence, and may suggest pharmacotherapeutical targets for treating ethanol addiction.

In recent years, several reports have suggested a close interaction between the endogenous cannabinoid system and consumption of ethanol. For instance, administration of SR-141716 A, a cannabinoid receptor antagonist, inhibits sucrose and ethanol intake (Arnone et al., 1997; Freedland et al., 2001), reduces voluntary ethanol intake in ethanol-prefering rats (Colombo et al., 1998) and decreases ethanol self-administration in rats exposed to ethanol-vapour chambers (Rodríguez de Fonseca et al., 1999). On the other hand, chronic ethanol administration in mice down-regulates cannabinoid receptor binding (Basavarajappa et al., 1998) and GTPγS binding in brain synaptic membranes (Basavarajappa and Hungund, 1999). Furthermore, it has been suggested that endocannabinoid signalling acting at CB₁ receptors is involved in ethanol preference (Hungund et al., 2003; Wang et al., 2003). However, little is known about the effects of chronic ethanol consumption on the endogenous cannabinoid CB₁ receptor in specific brain regions. Therefore, the purpose of this study was to examine, using in situ hybridization histochemistry, the effects of chronic ethanol consumption on cannabinoid CB₁ receptor gene expression in selected areas of the rat brain.

MATERIALS AND METHODS

Animals

Male Wistar rats (age 3 months, 275–300 g body weight, at the beginning of the experiment) obtained from Harlan (Barcelona, Spain) were used in this study. All animals were maintained in controlled temperature (23 ± 1°C) and light (lights on between 08.00 and 20.00 hours) conditions. All experiments were performed following the highest standards of animal care, monitoring health and minimizing pain and suffering, in accordance with National and International Laws for the Care and Use of Laboratory Animals.

Ethanol intake protocol

Rats were divided in two groups: (1) Rats with unlimited access to water containing saccharin (0.25% w/v); and (2) rats with unlimited access only to a solution of ethanol (10% v/v) containing saccharin (0.25% w/v) for a total period of 52 days. This time period was chosen when stable (low variability) ethanol intake (7–8 days) was achieved. Both groups of rats received food pellets ad libitum. Ethanol intake was measured every two days, and results of ethanol consumption were expressed in grams of ethanol per kg per day. At the end of this period, rats were killed by decapitation and their brains were quickly removed and frozen over dry ice.

In situ hybridization histochemistry

Coronal brain sections were cut at 12 μm at three different levels containing the regions of interest: caudate-putamen (CPu) and cingulate cortex (Ccg), ventromedial hypothalamic nucleus (VMN), CA1, CA2, CA3 fields of hippocampus and dentate gyrus (DG). All the sections were obtained according
to the Paxinos and Watson ‘Atlas’ (1998). In situ hybridization histochemistry (ISHH) was performed as described previously (Young et al., 1986) using synthetic oligonucleotide probes complementary to cannabinoid CB₁ receptor (CB₁) mRNA (bases 4–51, 349–396, 952–999) (Corchero et al., 1999). Briefly, oligonucleotide probes were labelled using terminal deoxynucleotidyl transferase (Boehringer, Madrid, Spain) to add a ³⁵S-labelled deoxyATP (1000 Ci/mmol; Amersham, Madrid, Spain) tail to the 3’ end of the probes. The probes (in 50 μl of hybridization buffer) were applied to each section and left overnight at 37°C for hybridization. Following hybridization, sections were washed four times for 15 min each in 0.15 mol/l NaCl, 0.015 mol/l sodium citrate, pH 7.2 (1× saline sodium citrate, SSC) at 55°C, and this was followed by two 30-min washes in 1× SSC at room temperature, one brief water dip and a blow-dry with air. In order to control for imaging enhancement variables, each set of slides was apposed to the same film (Kodak BioMax MR-1; Amersham, Madrid, Spain) in individualized cassettes for 15 days.

Image analyses quantification

Autoradiograms from in situ hybridization studies were analysed with a PC computer using the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). Optical densities were calculated from the uncalibrated mode of the Image program by subtracting from each measurement its corresponding background and expressed in grey scale units. The background measurement was taken from an area of the slice with the lowest nonspecific hybridization signal and subtracted from the hybridization signal measurement in the same slice. Measurements were pooled from brain sections and the values were averaged. Results were presented considering mean control values as 100%.

Statistical analyses

Statistical analyses were performed using Student’s t-test to compare control and ethanol 10% (v/v) groups. Differences were considered significant if the probability of error was less than 5%.

RESULTS

The average intake of rats having unlimited access to ethanol (10% v/v) containing saccharin (0.25% w/v) was 5.8 ± 0.1 g ethanol per kg per day (Fig. 1). This mean was calculated from 208 measurements over the entire period of 52 days. No
DISCUSSION

The results of the present study clearly indicate that forced consumption of high levels of ethanol significantly decreased cannabinoid CB1 receptor gene expression in most of the selected brain areas examined. Furthermore, these findings strongly suggest that cannabinoid receptors play an important role in the development of ethanol dependence, and may be considered as an alternative therapeutic target for the design of new anticraving drugs.

The decrease in cannabinoid CB1 gene expression in various brain regions of rats found in our study is in agreement with Basavarajappa et al. (1998) and Basavarajappa and Hungund (1999), who showed that chronic ethanol administration resulted in down-regulation of CB1 receptors and that CB1 receptor agonist stimulated GTPγS binding in mouse synaptic plasma membranes. However, González et al. (2002), studying the effects of chronic ethanol intake, found no alterations in CB1 receptor binding or CB1 receptor gene expression. The explanation for this discrepancy may be found in differences between the two experimental designs. For instance, the average of ethanol intake in our study (5.94 g ethanol per kg per day) was much higher than those reported by González et al. (2002) [0.25 ml ethanol (7.2%) per kg weight per day, approximately 0.014 g/kg weight/day, considering that the density of ethanol is 810 g/l and taking into account the average weight of the rats used in the experiment]. This is probably due to the fact that in our study we used saccharin (0.25% w/v) to counteract rodents’ natural aversion to ethanol 10% (v/v), thus potentially increasing the daily amount of ethanol drunk. In addition, the time that rats were exposed to ethanol in our study was more than three times longer (52 vs. 15 days) than the period chosen by González et al. (2002). Given the differences found between the two studies, it is tempting to speculate that prolonged daily exposure to high levels of ethanol intake may be necessary for reducing cannabinoid receptor function.

The precise mechanism through which prolonged ethanol intake decreases CB1 receptor mRNA levels remains to be clarified. However, it is possible to hypothesize that an increase in brain endocannabinoid ligands accounts for down-regulation of the cannabinoid receptor. In general, it is well accepted that receptors will undergo desensitization (down-regulation) when they are continuously activated by their endogenous ligands. Thus, down-regulation of CB1 receptor mRNA levels may occur because of increased levels of AEA and 2-AG resulting from chronic ethanol consumption. It is clearly shown using neuronal cells that chronic ethanol increases both AEA and 2-AG (Basavarajappa and Hungund, 1999; Basavarajappa et al., 2000; 2003). Alternatively, ethanol consumption may have a direct effect on CB1 gene expression or may inhibit CB1 receptor mRNA levels via an indirect mechanism not related to altered release of endogenous cannabinoid ligands.

The fact that alterations in cannabinoid gene expression occurred after prolonged exposure to ethanol intake supports the notion that manipulations of the cannabinoid CB1 system may affect ethanol intake. Indeed, administration of cannabinoid receptor agonists such as WIN-55,212–2 and CP-55,940 increased voluntary ethanol intake in Sardinian ethanol-prefering (sP) rats (Colombo et al., 2002), whereas the cannabinoid receptor antagonist SR-141,716A decreased voluntary ethanol intake in C57BL/6 (Arnone et al., 1997), ethanol self-administering Long Evans rats (Freedland et al., 2001) and Sardinian ethanol-prefering (sP) rats (Colombo et al., 1998).

The present study shows the first evidence of regional decrease in cannabinoid CB1 receptor gene expression in the rat brain after prolonged exposure to ethanol intake. The brain areas...
examined in the present study (CPu, VMN, Ccg, and hippocampal nuclei) were selected because of their potential role in the neurobiological mechanisms involved in drug dependence (González et al., 2002; Herrera et al., 2003; Tang et al., 2003).

CPu has been linked to motor and emotional behaviour responses to withdrawal syndromes induced by drugs of abuse (Oliva et al., 2003). Alterations in opioid gene expression in the CPu have also been related to high vulnerability to opiates (Cowen et al., 1998; Martin et al., 1999). VMN exhibits the highest levels of cannabinoid receptor and cannabinoid receptor gene expression in the hypothalamus of the rat (Herkenham et al., 1990) and regulates ingestive (Jamshidi and Taylor, 2001) and reproductive behaviours (Li et al., 1997), both of which are known to be altered in ethanol dependence. The hippocampus is the main brain nucleus in which cannabinoids probably exert their effects on memory and cognition (Herkenham et al., 1990; Ameri, 1999). Activation of cannabinoid receptors in the hippocampus inhibits neurotransmitter release (acetylcholine, GABA, glutamate and norepinephrine) (Doherty and Dingle, 2003), which presumably contributes to the short- and long-term plasticity underlying memory alterations that often occur after prolonged exposure to ethanol. The results of long-term alterations in cannabinoid receptor gene expression during chronic ethanol consumption in the hippocampal fields may be related to alterations in learning and memory occurring in ethanol-dependent subjects. Indeed, recent work has posited common molecular and cellular substrates of addiction and memory, suggesting a close relationship between brain circuits involved in the regulation of drug dependence and learning and memory (Nestler, 2002).

In conclusion, the results of this study revealed lower cannabinoid receptor function in CPu, VMN, CA1 and CA2 areas of the hippocampus in rats after prolonged exposure to forced ethanol intake. These findings suggest that cannabinoid CB₁ receptors play an important role in the neurochemical mechanisms involved in ethanol consumption and strongly support the potential relevance of cannabinoid CB₁ receptors in new pharmacological strategies for treating ethanol dependence.

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