



Long Term Effect of Ethanol on GABA-Benzodiazepine Receptors of Rat Brain Regions

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Research Article

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Abstract

Objective: The aim of this study is to investigate the effects of chronic exposure of ethanol ingestion on GABA benzodiazepine receptor complex in four rat brain regions.

Methods: Male adult rats from a colony were housed with food and drinking water / ethanol (85%+15% v/v). After 13 months of chronic ethanol consumption, the rats were killed by decapitation and the brain regions dissected and the crude synaptosomal fraction (P2) was prepared in ice-cold 0.32 M sucrose. The resulting pellet was resuspended in Tris-HCl buffer (pH 7.4). The samples were kept frozen at -20 °C until binding determination.

Results: Long term ethanol ingestion by the rat resulted in a decrease K_d values in the striatum and hippocampus, and an increase of K_d values in the cerebellum and frontal cortex. Long term ethanol consumption had no statistically significant effect on GABA-benzodiazepine binding (B_{max}), but a trend toward a reduction in the all brain regions studied. The different responses of GABA receptor affinity of the brain regions to chronic ethanol ingestion may result from quantitative differences in the distribution of GABA-ergic neurons in the tested brain areas.

Conclusion: The increase in the affinity of the striatum and hippocampus and the decreased affinity of cerebellum and frontal cortex, by long term continuous ethanol ingestion, may result from quantitative differences in the distribution of GABA-ergic neurons in the tested brain regions.

Key words: GABA receptors, Brain regions. Chronic ethanol, GABA benzodiazepine receptors

INTRODUCTION

Repeated exposure to ethanol causes a variety of complex changes in the central nervous system (CNS), some of which are suggested as behavioral tolerance and physical dependence (1,2 and 3). Although the underlying mechanisms responsible for CNS changes associated with ethanol ingestion are unclear, previous studies suggest the involvement of several neurotransmitter receptor systems, including the GABA receptor complex (4,5). A number of studies in the literature have described the effects of ethanol on the GABA-benzodiazepine receptors, sometimes with conflicting results (6, 7 and 8). In particular, in vitro studies have varied from no effect (9) to stimulation of diazepam binding to the brain membranes (10). Similarly, differential effects on the GABA binding have also been observed after a single intraperitoneal dose of ethanol (11). Interpretation of these results, however, is difficult in part due to the complexity and heterogeneity of the brain and treatment protocols, as well as the multiple actions of ethanol in vivo. Furthermore, chronic ethanol ingestion in man is considered as a long-term continuous process and the behavioral changes might only result in the chronic exposure to ethanol (12, 13 and 14). Since GABA receptors are unevenly distributed in frontal cortex, hippocampus, striatum and cerebellum (15), in the present work we investigated how long-term ethanol consumption could affect the GABA benzodiazepine binding in the frontal cortex, hippocampus, striatum and cerebellum of rat brain. The results reveal marked differences in the binding of the brain areas of rats exposed to ethanol for 13 months.

MATERIAL AND METHODOLOGY

Materials:

[N-methyl-3H] flunitrazepam, 84 Ci/m.mol, was purchased from Amersham. Clonazepam was donated by F. Hoffman-La Roche Ltd, Basel, Switzerland. All other reagents used were of ANALAR grade (or the highest grade available) unless stated otherwise and made up in double distilled water.



Membrane preparation:

Male adult Wistar rats from a colony bred in our laboratory were used. Animals were maintained with respect to the animal welfare regulation in animal house under a 12 hour light-dark cycle (light on 07.00, local time). For chronic ethanol treatment rats were given food and drinking water /ethanol (85% +15% v/v), ad libitum and for control group, food and drinking water (no ethanol) were accessible ad libitum. After 13 months of chronic ethanol consumption, the rats were killed by decapitation and main brain regions dissected over an ice plate, by the method of Glowinski and Iversen (17). Briefly, cerebellum was separated forebrain, and the striatum, including caudate nucleus, putamen nucleus and globus pallidus was dissected out from the frontal cortex, then hippocampus was separated. The crude synaptosomal fraction (P2) was prepared essentially as described by Messripour and Clark (18). Pooled dissected brain regions from six rat brain was homogenized in ice-cold 0.32 M sucrose and centrifuged (4°C) at 1000 g and the supernatant was centrifuged (4°C) at 30000 g. The resulting pellet was washed 3 times with Tris-HCl buffer pH 7.4 and resuspended in 1 ml of the same buffer. The samples were kept frozen at -20 °C until binding determination. Protein in the homogenized frozen-thawed sample was measured by the method of Lowry et al. (19).

Binding Assay

Flunitrazepam is a well known benzodiazepine and has high affinity for the benzodiazepine receptor that is associated to GABA_A receptors (20). [3H] Flunitrazepam binding was carried out by incubating 0.5 ml aliquots of the homogenized P2 membrane (200 µg protein) with [3H] flunitrazepam in a range of concentrations of 0.50 µM to 10.00 µM in a final volume of 0.7 ml. The non-specific binding was determined in the presence of c1onazepam (about 5% of total bound). The assay tubes were incubated for 30 min in a crushed ice bath. The incubation was terminated by the addition of 5 ml ice-cold 50 mM Tris- HCl buffer (pH 7.4) and filtered under a constant vacuum through Whatman GF/B fiberglass discs. Incubation tubes and filters were washed three times with 5 ml of ice-cold buffer and treated with 6 ml ACS scintillation cocktail. The bound radioactivity was counted by a liquid scintillation counter. Specific binding was calculated by subtracting the non-specific binding from the total binding and was expressed as fmol/mg protein.

Data Analysis

The receptor affinity (Kd) and maximum binding capacity (B max) were estimated by the Eadie-Hofstee analysis. The standard deviation of the background in such estimation and the standard errors of B max and Kd were calculated according to Zivin and Waud (21), The statistical significance between Kd values was established by testing the common variance hypothesis, then the covariance analysis.

RESULTS

Specific binding of increasing concentrations of [3H] flunitrazepam to the membrane prepared from the brain regions was saturable (data not shown). Scatchard plots of specific [3H] flunitrazepam binding were linear. Table I shows the results of Scatchard analysis, using the samples from alcoholic rats after a 13 month exposure to ethanol and corresponding controls. The relative Kd values for the four different brain regions of the control were as follows; hippocampus> frontal cortex, > striatum> cerebellum. The relative binding capacities (B max) of the four brain regions were different and were as follows; frontal cortex> cerebellum> hippocampus> striatum. Kd values were decreased, in another words the receptor affinity is increased significantly in the striatum and hippocampus, but increased in the cerebellum and frontal cortex, of the ethanol-treated rats. While, long-term continuous ethanol consumption has no statistically significant effect, but a trend toward a reduction on the B max values,

Table I. Effect of chronic ethanol ingestion on specific binding of [3H] flunitrazepam in different regions of the rat brain.

Brain regions	Control rats		Alcohol-treated rats	
	Kd	B max	Kd	B max
Frontal cortex,	8.9 ± 1.6	356 ± 46	10.5 ± 1.6*	323 ± 35
Striatum	5.7 ± 0.8	183 ± 15	1.6 ± 0.1*	164 ± 5
Hippocampus	9.1 ± 2.4	216 ± 40	5.6 ± 0.9*	183 ± 17
Cerebellum	4.9 ± 0.	332 ± 17	6.4 ± 0.2*	313 ± 34

[3H] Flunitrazepam binding to previously frozen-thawed membrane was performed in 50 mM Tris-HCL buffer pH 7.4. Kd values are given as nM and B max values are given as fmol/mg protein. Values (means ± SD of four determinations) are the results of Scatchard analysis of saturation isotherms using [3H]-flunitrazepam concentrations of 0.5-10 µM. *Significantly different from the control value (P<0.05).

DISCUSSION

The present results indicated that long-term continuous treatment (13 months) with ethanol alters B max and the affinity of [3H] flunitrazepam in synaptosomal membrane in different rat brain regions. In contrast, Shanley et/al. (22) found no significant change in Kd or B max for [3H]



flunitrazepam in rat brain homogenates after three months of oral ethanol consumption. Although the reason for the relative increase in benzodiazepine receptor affinity in the striatum and hippocampus, and also the decrease in the receptor affinity in the cerebellum and frontal cortex, after long term ethanol ingestion is unclear; it may be explained by the regional specific changes in the regional function or the amount of GABA. The concentration of GABA has been shown to be higher in the striatum and hippocampus as compared with the frontal cortex, and cerebellum [5]. In addition, several lines of evidence indicated that the GABA receptor subunits were differentially expressed in the rat brain, suggesting the existence of different subtypes of the GABA receptor in different brain regions (23, 24). Furthermore, it has been reported that GABA receptors are unequally distributed in the brain regions and the receptors are highly localized in striatum and hippocampus as compared to other areas (15). The increase in the benzodiazepine receptor affinity observed in the striatum and hippocampus would suggest that long term ethanol consumption may enhance GABA agonists' binding affinity and then facilitate GABA transmission in these areas. The different response of GABA receptor affinity of the brain regions to the long term ethanol ingestion may result from differences in the distribution of GABA-ergic neurons in the tested brain areas. The selective increase in benzodiazepine receptor affinity in the brain regions induced by the long term treatment with ethanol has important implications with respect to the inhibitory nature of GABA transmission (6, 7). It has been demonstrated that the GABA-ergic neurons seem to be mediated by at least two pharmacologically distinct benzodiazepine receptor subtypes; a GABA_A receptor enriched in cerebellum, and a GABA_B receptor enriched in hippocampus and some other brain regions (25, 26). It is therefore possible that long-term ethanol ingestion leads to different alternations of GABA-benzodiazepine receptor affinity in different brain regions. It is concluded that long-term continuous ethanol treatment modulates GABA-benzodiazepine binding, so that the altered efficacy of the receptor complex in different brain regions leads to altered behavior of GABA-benzodiazepine receptor complexes in the brain.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.