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THE EFFECTS OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION AND ITS WITHDRAWAL ON γ-AMINOBUTYRIC ACID RECEPTOR BINDING IN RAT BRAIN

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1 The effects of acute and chronic ethanol administration, and withdrawal on the binding of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), was investigated in rat brain.

2 Acute ethanol (2 to 4 g/kg i.p. 30 min before removal of brain) produced an increase in the binding capacity of the low affinity GABA receptor binding site.

3 Following chronic ethanol administration (1 to 21 days), the GABA receptor binding characteristics were not altered. These results suggest a possible adaptation of GABA receptors to the continuous presence of ethanol at the GABA synapse.

4 During ethanol withdrawal, the affinity of the low affinity GABA receptor binding site was significantly lower than pair-fed controls at 8 and 16 h withdrawal.

5 These results suggest that GABA receptor sensitivity may play a role in some of the neuropharmacological effects of ethanol and in its withdrawal symptoms.

Introduction

The mechanism of action of ethanol, and the neuronal components involved in its tolerance, physical dependence and withdrawal are not known. Since ethanol is known to affect membrane fluidity (Seeman, 1972; Hill & Gangham, 1975), a clearer understanding of the molecular interaction between ethanol and various facets of synaptic transmission is essential to our understanding of ethanol-induced aberrations in the central nervous system (CNS). A variety of neuronal pathways have been implicated in various states of alcoholism (Goldstein, 1973; Kalant, 1974). Furthermore, since ethanol is a CNS depressant, and hyperexcitability seizures are observed during its withdrawal (Victor, 1970; Goldstein, 1973), it is reasonable to assume that ethanol may produce these symptoms by altering the balance between excitatory and inhibitory synaptic activity. Thus, ethanol has been reported to affect dopamine receptor (Tabakoff & Hoffman, 1978), glutamate receptor (Michaelis, Mulvaney & Freed, 1978) and adrenoceptor (Banerjee, Sharma & Khanna, 1978) systems.

It is widely accepted that γ-aminobutyric acid (GABA) is a major pre- and postsynaptic inhibitory neurotransmitter in the mammalian CNS (Curtis & Johnston, 1974; Krnjević, 1974). Several lines of evidence have implicated GABA system in the actions of ethanol and in its tolerance and withdrawal (Banna, 1969; Goldstein, 1973; Davidoff, 1973; Sutton & Simmonds, 1973; Leitch, Cott, Carlsson, Engel & Lindquist, 1976; Backer, Siegman & Guthrie, 1977). In addition ethanol has been reported to enhance GABAergic tranmission (Banna, 1969; Davidoff, 1973; Cott *et al.*, 1976; Biswas & Carlsson, 1977). Furthermore, GABA mimetics have been reported to reduce ethanol withdrawal symptoms, while GABA antagonists produce symptoms similar to those seen during ethanol withdrawal (Goldstein, 1973; Biswas & Carlsson, 1977). These effects of ethanol could be mediated by an interaction between ethanol with any one or more of presynaptic (uptake, release, synthesis) or postsynaptic (receptor, ionophore) events at the GABA synapse. In view of these observations the effects of acute and chronic ethanol administration and its withdrawal on GABA receptor binding in rat brain have been investigated.

Methods

Male Sprague–Dawley rats (150 to 170 g) were housed two per cage at 22 ± 1°C on a 12 h light-dark cycle, and had free access to laboratory chow and water until the beginning of the experimental protocol. [^{3}H]-GABA was purchased from Amersham (Arlington Heights, Ill., U.S.A.). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

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Acute ethanol treatment

For acute studies, ethanol was administered at a dose of 1 to 4 g/kg body wt. by intraperitoneal (i.p.) injection as a 20% (v/v) solution. Control rats received equicalories of sucrose in 0.9% w/v NaCl solution (saline). Animals were decapitated 30 min after the i.p. injection, the brains excluding the brain stem caudal to the cerebellum, were rapidly removed and processed for tissue preparation as described below.

Chronic ethanol treatment

Chronic ethanol was administered as a 7% solution in liquid diet (chocolate flavoured Sustacal from Mead-Johnson). Rats were initially acclimatized to the control liquid diet containing Sustacal (1 cal/ml), 3 g/l vitamin fortification mix (ICN) and 96.8 g/l sucrose. Following one day on control-Sustacal diet, pair-fed controls were continued on this diet, while ethanol-treated animals were given a similar liquid diet in which sucrose was replaced with 59.6 g/l ethanol (ethanol-Sustacal diet). Daily calorie intake of pair-fed control rats was restricted to equal the mean calorie intake of the ethanol consuming rats. This protocol is similar to that used by other investigators (e.g. Ritzman & Tabakoff, 1976). For chronic ethanol studies, pair-fed controls and ethanol-treated rats (3 to 5 in each group) were killed on days 1, 3, 6, 7, 9 and 14, after being on the liquid diet.

For withdrawal studies, rats were chronically treated with ethanol-Sustacal diet for 21 days and were then given control-Sustacal diet, as described above. Animals were killed at 0, 8, 16, 24 and 72 h.

Withdrawal reaction

Withdrawal reaction was assessed by subjecting individual rats to audiogenic seizures by the jangling keys method of Dice (1935). The symptoms were scored as follows: no response = 0; hyperactivity and wild running = 2; clonic seizure = 3; tonic–clonic seizure = 4.

Tissue preparation

Following acute and chronic ethanol treatments and, at various times after withdrawal from ethanol, rats were decapitated, brains (excluding the brain stem caudal to the cerebellum) rapidly removed and kept in iced 0.32 M sucrose. The tissue was prepared as described elsewhere (Olsen, Ticku, VanNess & Greenlee, 1978; Ticku, 1979). Briefly, brains were homogenized in 0.32 M sucrose and centrifuged at 580 *g* for 10 min; the supernatant was centrifuged at 100,000 *g* for 45 min to obtain the crude mitochondrial plus microsomal ($P_2 + P_3$) fractions. The fractions were osmotically shocked in iced distilled water, repelleted, osmotic shock treatment was repeated, and the pellet resuspended in 0.05 M Tris citrate buffer pH 7.1 and frozen at −20°C overnight. The tissue was thawed at room temperature, pelleted, homogenized in 0.05 M Tris citrate, repelleted, resuspended in the buffer and frozen. On the day of the assay, the samples were thawed, repelleted, washed once in the buffer and finally resuspended in the same buffer at a protein concentration of 0.8 to 1.2 mg/ml. The freeze-thaw and excessive washing of the tissue is necessary to eliminate the uptake sites, and endogenous inhibitors of GABA binding (Greenlee, VanNess & Olsen, 1978; Toffano, Guidotti & Costa, 1978). These procedures also eliminate the need for using Triton X-100 (Ticku & Burch, unpublished observations). Protein was estimated according to the method of Lowry, Rosebrough, Farr & Randall (1951). All the tissue preparation was done at 0 to 4°C and in Na^+-free buffer.

GABA binding

The binding of [^{3}H]-GABA to its receptor-like sites was assayed by a modification of the method of Zukin, Young & Snyder (1974) and Enna & Snyder (1975), by a centrifugation assay, as described previously (Olsen *et al.*, 1978; Ticku, 1979). Binding assays were performed by incubating, in triplicate, aliquots of $P_2 + P_3$ homogenate (0.7 to 1.0 mg) with [^{3}H]-GABA (66 Ci/mmol) without or with excess nonradioactive GABA (0.1 mM) for 5 min at 0 to 4°C in 1 ml volume in scintillation Biovials. The concentration of [^{3}H]-GABA was varied for the low concen-

Table 1 Effect of acute ethanol administration on [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) binding to rat brain membranes

	Specific GABA binding (fmol/mg protein)
Control (n = 3)	75.0 ± 8.8
Ethanol:	
1 g/kg (n = 3)	72.6 ± 5.2
2 g/kg (n = 4)	90.3 ± 3.4*
4 g/kg (n = 4)	88.1 ± 5.3†

Rats were decapitated 30 min after an injection of ethanol (i.p. as 20% v/v solution) or saline, and $P_2 + P_3$ fraction was prepared as described in the Methods. GABA binding was studied by a centrifugation assay, using 2 nM [^{3}H]-GABA (66 Ci/mmol) and background was determined in presence of 0.1 mM non-radioactive GABA. Values represent the mean ± s.d. of the number of experiments (in triplicate) given in parentheses.
* $P < 0.05$, when compared to controls; † $P < 0.1$, when compared to controls.

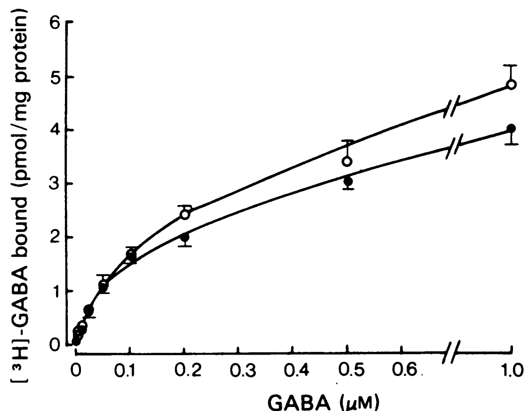

Figure 1 Effect of acute ethanol (4 g/kg) administration on the binding of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) to rat brain membranes. Ethanol (20% v/v) or equivalent calories of sucrose in saline (to controls) was administered by i.p. injection and animals killed 30 min later. The crude mitochondrial plus microsomal fractions of control (●) or acutely treated (○) rat brains were incubated with various concentrations of [^{3}H]-GABA for 5 min at 0°C and assayed by a centrifugation assay as described in Methods. The concentrations of [^{3}H]-GABA (66 Ci/mmol) were varied from 0.25 to 1004 nM as described in Methods and the legend to Table 2. Background was obtained in the presence of 0.1 mM nonradioactive GABA, and has been subtracted from each point. Each point is the mean ± s.d. of four experiments done in triplicate.

tration points (0.1 to 4 nM) and for the high points (7 to 1004 nM), the concentration of nonradioactive GABA was varied, keeping [^{3}H]-GABA constant at 4 nM. Following incubation, the vials were centrifuged at 48,000 g for 10 min. The supernatant was discarded, pellets rapidly rinsed with iced buffer. Pellets were solubilized overnight with 0.3 ml Soluene-350 (Packard) and radioactivity counted in 3 ml of toluene containing 5 g/l of 2,5-diphenyloxazole. The counting efficiency, as estimated by internal standard ([^{3}H]-toluene) method was 40 ± 1%.

The specific binding obtained by subtracting the background from the total pelleted radioactivity, i.e. the amount not displaced by excess (0.1 mM) non-radioactive GABA, is converted into pmol/mg protein bound. Data were analyzed by Scatchard plots and the affinity constants (K_D) and binding capacities (B_{max}) were obtained by computer drawn linear-regression of the binding data. Statistical analysis was by Student's t test.

Results

Effects of acute ethanol on GABA binding

Table 1 shows the effect of acute ethanol (1 to 4 g/kg) administration on the binding of GABA to rat brain membranes. A dose of 1 g/kg ethanol had no significant effect on GABA binding. However, acute doses of 2 and 4 g/kg produced a significant increase in the GABA binding. At 2 g/kg, the specific binding was increased from a control value of 75 ± 8.8 fmol/mg to 90.3 ± 3.4 fmol/mg ($P < 0.05$). A dose of 4 g/kg also produced a similar (but no further) increase in the binding activity.

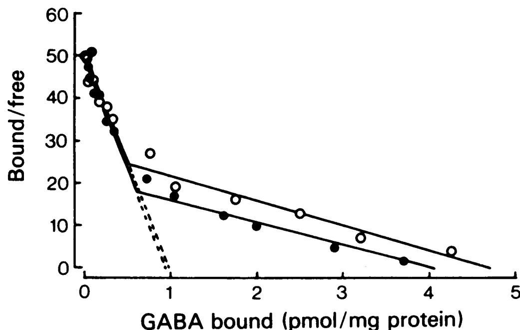

Figure 2 Scatchard plot of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) binding from control (●) and ethanol (4 g/kg)-treated (○) rat brain membranes. Data were analyzed by computer-drawn linear regression of the Scatchard plot. Each point is the mean of one experiment (done in triplicate) and was replicated four times.

Figure 1 shows the effect of 4 g/kg ethanol on the GABA binding saturation isotherm (values are mean ± s.d. of four experiments). Figure 2 shows the Scatchard plot of one such experiment. The high and low affinity GABA binding sites appear parallel and similar in both control and ethanol (4 g/kg)-treated animals, suggesting no apparent change in the binding affinities of the two GABA receptor sites. However, acute ethanol administration appears to enhance the capacity of low affinity GABA receptor sites. Table 2 summarizes the effects of acute ethanol administration (1 to 4 g/kg) on the affinities (K_Ds) and the binding capacities (B_{max}) of the two GABA receptor sites. The major effect of acute administration of ethanol at 2 to 4 g/kg doses was an increase in the binding capacity of the low affinity site (B_{max_2}). Thus, 2 g/kg increased the B_{max_2}, from a control value of 4.124 ± 0.39 pmol/mg to 4.893 ± 0.25 pmol/mg (14% increase; $P < 0.02$), while 4 g/kg increased it to 4.780 ± 0.14 pmol/mg (12% increase, $P < 0.05$). A dose of 1 g/kg ethanol had no effect on the GABA binding constants.

Ethanol intake

Treated rats drank an average of 45 ± 9 ml/day of ethanol-Sustacal diet. This corresponds to a daily ethanol intake of 11 to 17 g/kg per rat. Body weights were not significantly different in control or ethanol-

treated Sustacal diet groups at the end of various treatments.

GABA saturation curves were identical in Sustacal-diet rats and laboratory-chow fed rats. Scatchard analysis of GABA binding from both laboratory-chow fed and Sustacal-fed rats gave two classes of binding sites. The affinities and the binding capacities in the laboratory-chow fed rats (K_{D1} = 26 ± 6 nM; K_{D2} = 139 ± 12 nM; B_{max_1} = 1.23 ± 0.22 pmol/mg; B_{max_2} = 4.19 ± 0.35 pmol/mg, $n = 4$) and Sustacal-fed rats (K_{D1} = 21 ± 6 nM; K_{D2} = 147 ± 19 nM; B_{max_1} = 1.12 ± 0.15 pmol/mg; B_{max_2} = 4.01 ± 0.43 pmol/mg, $n = 9$) were similar. Thus, Sustacal diet, by itself, does not appear to alter GABA receptor characteristics.

Effects of chronic ethanol administration on GABA receptor sensitivity

The effect of chronic ethanol administration on the affinities and binding capacities of GABA receptor sites was investigated after 1, 3, 6, 7, 9 and 14 day treatments. Pair-fed control rat brains gave similar binding characteristics following 1, 3, 7 and 14 day treatments on control-Sustacal diet (data not shown). The control values in Table 3 are mean ± s.d. of combined pair-fed control groups. Control Sustacal-diet fed rats bound GABA to two classes of sites with K_{D1} of 21 ± 6 nM, K_{D2} of 147 ± 19 nM and binding capacities of 1.12 ± 0.15 pmol/mg and 4.006 ± 0.43 pmol/mg, respectively. These values are in agreement

Table 2 Effect of acute ethanol administration on [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) receptor sensitivity in rat brain

Treatment	K_D (nM) K_{D1}	K_{D2}	B_{max} (pmol/mg protein) B_{max_1}	B_{max_2}
Control	25 ± 6	136 ± 11	1.15 ± 0.07	4.124 ± 0.39
1 g/kg	26 ± 5	125 ± 9	1.02 ± 0.11	4.144 ± 0.31
2 g/kg	22 ± 9	144 ± 12	1.24 ± 0.44	4.893 ± 0.25*
4 g/kg	23 ± 5	151 ± 15	1.10 ± 0.26	4.780 ± 0.14†

Rats were acutely treated with 1 to 4 g/kg ethanol or corresponding volumes of saline (controls) and decapitated 30 min later as described in the Methods. Tissue preparation and binding assays were performed as described in the text. Concentration of [^{3}H]-GABA was varied for first five points (0.25 to 4 nM; 66 Ci/mmol) and for another eight points (7 to 1004 nM) a constant concentration of 4 nM [^{3}H]-GABA (66 Ci/mmol) was diluted with non-radioactive GABA. Each point was determined in triplicate with simultaneous background determined in presence of 0.1 mM nonradioactive GABA. Data were analyzed by computer-drawn linear regression of the Scatchard data. Results are the mean ± s.d. of four experiments for each group.
* $P < 0.02$; † $P < 0.05$; when compared to controls.

Table 3 Effect of chronic ethanol administration on the affinities and binding capacities of γ-aminobutyric acid (GABA) receptor sites in rat brain

	K_D (nM) K_{D1}	K_{D2}	B_{max} (pmol/mg protein) B_{max_1}	B_{max_2}
Control (9)	21 ± 6	147 ± 19	1.12 ± 0.15	4.006 ± 0.43
1-day treatment (3)	24 ± 10	116 ± 21	0.97 ± 0.16	3.822 ± 0.29
3-day treatment (4)	28 ± 7	133 ± 21	1.14 ± 0.24	4.293 ± 0.19
6-day treatment (4)	17 ± 8	131 ± 19	1.02 ± 0.17	4.354 ± 0.43
7-day treatment (5)	20 ± 5	136 ± 16	0.78 ± 0.26	3.927 ± 0.39
9-day treatment (3)	19 ± 5	122 ± 28	1.21 ± 0.19	4.282 ± 0.15
14-day treatment (5)	21 ± 7	175 ± 27	0.84 ± 0.32	4.361 ± 0.38

Rats were administered control–Sustacal diet or ethanol–Sustacal diet for 1 to 14 days, killed and the tissue prepared as described in Methods. GABA binding was studied and analyzed as described in Methods and legends to Table 2. The values are the mean ± s.d. of the number of experiments (each in triplicate) given in parentheses.

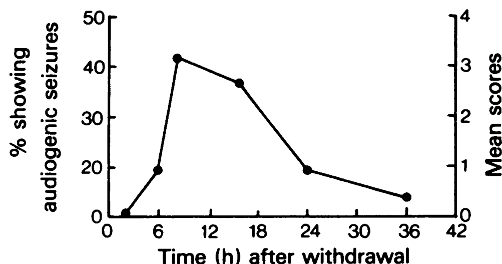

Figure 3 Time course and mean audiogenic seizure score following withdrawal for rats 3 week chronically treated with ethanol. Rats were scored individually as described in Methods, and for each point, 4 to 6 rats were used. Audiogenic seizures were elicited by jangling keys for 3 s by the method of Dice (1935).

with previously published results for mammalian brain (Enna & Snyder, 1977; Olsen, Ticku, Greenlee & VanNess, 1979; Ticku, 1979). Table 3 shows that chronic ethanol treatment for 1 to 14 days did not significantly alter either the affinities or the binding capacities of the two GABA receptor sites. K_{D2} value was lower than controls in 14 day ethanol-treated group; however, the difference was not statistically significant, due to large variation in this group.

Audiogenic seizure activity during withdrawal from ethanol

Our preliminary studies indicated that most rats did not exhibit audiogenic seizure activity during withdrawal, following 7 day chronic ethanol intake. However, rats that had been chronically treated with ethanol–Sustacal diet for three weeks did exhibit withdrawal symptoms. Figure 3 shows the mean audiogenic seizure score frequency and the time course of audiogenic seizures. Maximal audiogenic seizure activity was observed at 8 to 16 h after withdrawal, and little or no seizure activity was observed after 24 or 36 h.

Effect of ethanol withdrawal on GABA receptor sensitivity

The effects of withdrawal at various times after chronic ethanol treatment for 21 days, on GABA binding parameters in ethanol-treated and pair-fed controls is summarized in Table 4. Table 4 shows that GABA receptor affinities and binding capacities were not significantly different at 0 h withdrawn (i.e., chronic treatment) in treated and pair-fed animals. However, at 8 and 16 h withdrawal, ethanol-treated groups had significantly decreased affinity of the low affinity site (K_{D2}). Thus, while the K_{D2} of pair-fed control group was 127 ± 11 nM, 8 h post-withdrawal the K_D was shifted to 189 ± 14 nM ($P < 0.01$). Similarly, the K_{D2} of the 16 h withdrawal group was shifted from the pair-fed control value of 139 ± 15 nM, to 177 ± 12 nM ($P < 0.05$). At 24 h after withdrawal from Sustacal–ethanol diet, the affinity of the low affinity site (K_{D2}) returned to the control value (Table 4).

Discussion

GABA is a major inhibitory neurotransmitter in the

Table 4 γ-Aminobutyric acid (GABA) receptor sensitivity at various times after withdrawal from ethanol

Time after withdrawal	K_D (nM)		B_{max} (pmol/mg protein)	
	K_{D1}	K_{D2}	B_{max_1}	B_{max_2}
0 h Control (3)	22 ± 8	124 ± 13	1.12 ± 0.29	4.012 ± 0.35
Ethanol (5)	14 ± 5	136 ± 17	0.92 ± 0.34	4.324 ± 0.61
8 h Control (2)	19 ± 7	127 ± 11	1.03 ± 0.38	3.887 ± 0.52
Ethanol (4)	15 ± 6	189 ± 14*	0.77 ± 0.30	4.067 ± 0.68
16 h Control (2)	24 ± 6	139 ± 15	0.89 ± 0.25	4.256 ± 0.54
Ethanol (4)	18 ± 3	177 ± 12†	0.99 ± 0.18	4.242 ± 0.35
24 h Control (3)	17 ± 6	127 ± 19	1.23 ± 0.41	3.980 ± 0.35
Ethanol (4)	16 ± 4	141 ± 16	0.99 ± 0.22	4.296 ± 0.39
72 h Control (2)	26 ± 9	137 ± 9	0.87 ± 0.38	4.107 ± 0.55
Ethanol (3)	20 ± 8	129 ± 11	1.03 ± 0.34	3.863 ± 0.42

Ethanol-treated rats were given ethanol–Sustacal diet for 21 days and then changed to control-Sustacal diet for various times before they were killed. Pair-fed controls were given control–Sustacal diet throughout this period. Ethanol-treated and pair-fed control groups were killed at the times indicated and tissue was prepared as described in Methods. Results are the mean ± s.d. of the number of experiments (each done in triplicate) indicated in parentheses.

* $P < 0.01$, when compared to pair-fed control group; † $P < 0.05$, when compared to pair-fed control group.

mammalian CNS (Curtis & Johnston, 1974; Krnjević, 1974), and a variety of centrally acting drugs, like benzodiazepines (Polc, Mohler & Haefely, 1974; Costa, Guidotti & Toffano, 1978) and barbiturates (Nicoll, 1975; Ransom & Barker, 1976; Bowery & Dray, 1978), appear to affect GABAergic transmission. Ethanol, like barbiturates, is a CNS depressant and appears to have rather specific effects on GABA synapses (Miyahara, Esplin & Zablocka, 1966; Banna, 1969; Davidoff, 1973). Thus, ethanol has been found to enhance presynaptic inhibition in the cat spinal cord (Miyahara *et al.*, 1966), increase dorsal root potentials in frog spinal cord (Davidoff, 1973) and increase the peak and duration of postsynaptic inhibition in cuneate and gracilis nuclei of cats (Banna, 1969). The transmitter at these synapses appear to be GABA (Curtis & Johnston, 1974; Krnjević, 1974). Recently, it has been reported that ethanol selectively potentiated GABAergic transmission in feline cerebral cortex (Nestoros, 1979). Furthermore, bicuculline, a GABA synaptic antagonist, has been found to block the enhancing effects of ethanol on GABAergic transmission (Haefely, Polc, Schaffner, Keller, Pierri & Möhler, 1979). These lines of evidence suggest that ethanol may produce some of its neuropharmacological actions by affecting GABA-mediated pre- or postsynaptic events. Other lines of evidence also suggest a possible role for the GABA system in the production of ethanol dependence and withdrawal (Goldstein, 1973; Sutton & Simmonds, 1973; Leitch *et al.*, Hakkinen & Kulonen, 1976).

In the present investigation, it was found that a single dose of ethanol (2 to 4 g/kg) produces an increase in the binding capacity (B_{max_2}) of the low affinity site without significantly altering other GABA receptor binding parameters (Figures 1 and 2; Table 2). These results suggest that doses of ethanol which are known to produce CNS depression (2 to 4 g/kg) may produce a possible facilitatory effect on GABAergic transmission by increasing the density of the low affinity GABA binding sites. Although the effects of acute ethanol administration on B_{max_2} is not very pronounced, the consequences of this increase cannot be undermined, since GABA appears to be a transmitter at approximately 30 to 40% of the central synapses (Iversen & Bloom, 1972). The ability of ethanol to increase the density of the low affinity sites would be in agreement with the reported observations that GABA mimetics produce behavioural depression (Cott *et al.*, 1976) and that tolerance to ethanol was mimicked by picrotoxin and reduced by increasing GABA levels (Leitch *et al.*, 1977). Furthermore, the ability of ethanol to enhance GABAergic transmission (Miyahara *et al.*, 1966; Banna, 1969; Davidoff, 1973; Haefely *et al.*, 1979; Nestoros, 1979) may be due to the increase in the density of GABA receptor sites.

In contrast to the acute effects, animals chronically treated with ethanol did not exhibit any changes in either the affinities of the binding capacities of the two GABA receptor sites (Table 3), suggesting that adaptation may have developed at the GABA synapse to the continuous presence of ethanol. We are currently investigating the effects of chronic ethanol administration on GABA binding in discrete brain regions.

Since withdrawal from ethanol is known to produce hyperexcitability, and picrotoxin, a GABA synaptic antagonist, produces symptoms similar to ethanol withdrawal (Goldstein, 1973; Biswas & Carlsson, 1977), the GABA receptor sensitivity following withdrawal from ethanol was investigated. Table 4 shows that at 8 and 16 h after withdrawal, the affinity of the low affinity site (K_{D2}) was significantly lower than the pair-fed controls. This decrease in K_{D2} recovered with time, and at 24 h after withdrawal, ethanol and pair-fed groups gave identical binding characteristics (Table 4). The time course of changes in K_{D2} (Table 4) are in agreement with the audiogenic seizure activity during ethanol withdrawal (Figure 3). These results suggest that a depressed GABA synaptic function may be responsible for some of the withdrawal symptoms.

In summary, the results suggest an interaction between ethanol and brain membranes containing GABA receptors. These changes in receptor kinetics may be involved in some of the neuropharmacological effects seen after ethanol exposure. Although our results suggest the involvement of GABA receptors in ethanol effects, the possibility that other GABA synaptic events, especially GABA release and picrotoxin-sensitive sites at the GABA synapse, may also be altered by ethanol treatments and during its withdrawal cannot be ruled out. Earlier studies have demonstrated that picrotoxin, which binds at a site distinct from the GABA recognition site (Ticku, Ban & Olsen, 1978; Ticku, VanNess, Haycock, Levy & Olsen, 1978) is a target for depressant barbiturate and convulsant drug action (Ticku & Olsen, 1978; 1979). Studies are currently in progress to test the effects of ethanol on picrotoxin-sensitive sites at the GABA synapse.

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