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Effects of Chronic Ethanol Exposure on Adenylate Cyclase Activities in the Rat

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RABIN, R. A., R. C. BAKER AND R. A. DEITRICH. *Effects of chronic ethanol exposure on adenylate cyclase activities in the rat.* PHARMACOL BIOCHEM BEHAV 26(4) 693–697, 1987.—The effects of chronic ethanol administration on striatal and cerebellar adenylate cyclase systems were investigated in the rat. The chronic ethanol treatment resulted in behavioral tolerance, but no difference in the sensitivity of adenylate cyclase to *in vitro* ethanol was observed. In one set of experiments using 173–195 g rats, GTP-, dopamine- and NaF-stimulated adenylate cyclase activities in the striatum were higher in rats chronically treated with ethanol when compared to animals pair-fed the liquid diet. However, no difference in adenylate cyclase activity was observed in cerebellar or striatal tissues when larger rats (280–385 g) were used. In conclusion, an adaptive change in activation of adenylate cyclase by *in vitro* ethanol does not occur after chronic ethanol treatment. The observed changes in enzyme activity measured in the absence of *in vitro* ethanol do not appear to be a simple, direct effect of chronic ethanol treatment.

Chronic ethanol Tolerance Adenylate cyclase

REPEATED exposure to ethanol results in behavioral tolerance that is caused, in part, by a decrease in the sensitivity of the central nervous system to ethanol. The biochemical mechanisms responsible for this neuronal adaptation, however, are unclear. The acute actions of ethanol appear to be secondary to alterations in membrane properties [9,29], and after chronic ethanol administration the ability of ethanol to alter various membrane properties is attenuated. Thus, chronic ethanol treatment reduces the potency of ethanol to decrease membrane order [2, 7, 17], as well as decrease the potency of ethanol to inhibit Na-K ATPase activity [14,25], calcium uptake [8,13] and phospholipase A_2 activity [11].

The activity of the membrane-bound adenylate cyclase system, which mediates the actions of some neurotransmitters (see [3]), is increased by the *in vitro* addition of ethanol [6, 16, 22, 23, 32]. Alcohol-induced stimulation of adenylate cyclase activity, like the inhibition of Na-K ATPase activity and calcium uptake [24, 33, 34], appears to be secondary to an effect on a hydrophobic area of the membrane [5, 19, 21]. Little, however, is known about the effects of chronic ethanol exposure on the sensitivity of adenylate cyclase to *in vitro* ethanol. In rat cortex the sensitivity of basal adenylate cyclase to *in vitro* was not altered after a three week treatment with a liquid diet containing ethanol or after 48 hours of withdrawal [35]. Unfortunately, the effects of the chronic ethanol treatment on hormone-stimulated adenylate cyclase activity were not investigated. Another study using mouse striatal tissue reported a reduction in the sensitivity of basal adenylate cyclase activity to *in vitro* ethanol 24 hours after discontinuation of a seven day treatment with a liquid diet containing ethanol [10]. Although hormone-stimulated adenylate cyclase activity is more sensitive to the *in vitro* actions of ethanol [22,23], chronic ethanol treatment did not alter the sensitivity of dopamine-stimulated adenylate cyclase activity to *in vitro* ethanol [10]. The liquid diets used in the above studies were formulated, in part, on human rather than rodent nutrient requirements, and it is unclear what effects the liquid diet and the reduced food intake had on the adenylate cyclase system. In addition, the protocol used in one study [10] results in a significant reduction in body weight [26] and includes the complicating influence of ethanol withdrawal.

The present study was undertaken to determine the effects of chronic ethanol administration on striatal and cerebellar adenylate cyclase activities using a liquid diet [18] that was specifically formulated for rodents. Changes in intrinsic adenylate cyclase activity (i.e., activity measured in the absence of *in vitro* ethanol) as well as changes in enzyme sensitivity to *in vitro* ethanol were determined.

METHOD

Male, Sprague-Dawley rats (Part I: 173–195 g; Part II: 280–385 g) were randomly divided into groups and housed individually as described below. The ethanol-treated group received a liquid diet in which ethanol was isocalorically substituted for dextrin [18]. This liquid diet provides 67% of the calories as carbohydrate, 12% as fat and 21% as protein.

TABLE 1

BLOOD ALCOHOL LEVELS AT THE TIME OF REGAINING AERIAL RIGHTING REFLEX AND THE PERCENT INCREASE IN BODY WEIGHT IN RATS (INITIAL BODY WEIGHT 173–195 g) CHRONICALLY ADMINISTERED ETHANOL

	Blood Alcohol (mg %)	Body Weight (% increase)
Ethanol-treated	227 ± 12.3† (6)	62 ± 7.3* (6)
Pair-fed liquid diet	167 ± 8.2† (7)	80 ± 5.1* (7)
Pair-fed chow	177 ± 12.2 (7)	84 ± 2.1† (7)
Ad lib chow	187 ± 7.3 (7)	123 ± 3.0† (7)

*$p<0.05$, †$p<0.001$.

Data are expressed as mean ± S.E.M. for the number of animals in parentheses. Groups with the same superscripts are significantly different from each other. No other differences as outlined in the Method section are significant.

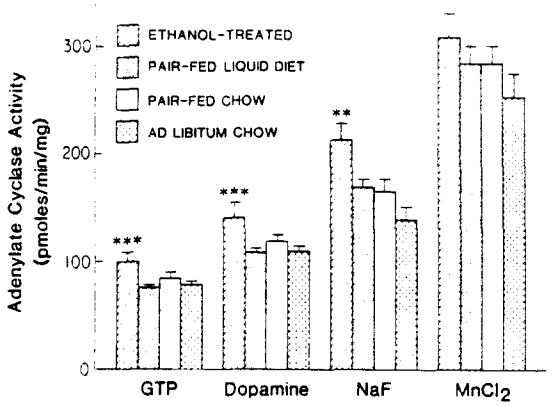

FIG. 1. Effects of chronic ethanol administration on striatal adenylate cyclase activity. Rats (initial body weight 173–195 g) were treated as described in the Method section. Adenylate cyclase activity was measured in the presence of 50 μM GTP, 50 μM dopamine plus 50 μM GTP, 10 mM NaF or 10 mM $MnCl_2$. Data are expressed as mean±S.E.M. for six ethanol-treated rats or for the 7 rats in each of the control groups. **$p<0.025$, ***$p<0.005$ comparing ethanol-treated animals to rats pair-fed the liquid diet. No other comparisons as described in the Method section were statistically significant.

The ethanol-treated group received the liquid diet with ethanol as 10% of total calories for three days, as 20% of total calories for the next three days and as 35% of total calories for the remainder of the 19 day treatment. Another group of rats (pair-fed liquid diet group) received the same number of calories consumed by the ethanol-treated group, but in the liquid diet without ethanol. A group of rats fed the liquid diet ad lib initially was included to determine the effects of reduced food intake, but rats allowed unrestricted access to the liquid diet gain an excessive amount of weight thereby negating their usefulness. The effects of a reduction in caloric intake were therefore investigated by including two additional groups of rats: one group (ad lib) received commercial lab chow (Wayne Rodent Blox) and water ad lib, and another group (pair-fed chow) received the same number of calories as consumed by the ethanol-treated group, but as lab chow. Pair-feeding of the animals with either the liquid diet or lab chow was done on a group basis: pair-fed animals received the average number of calories consumed by the whole ethanol-treated group. Food for all animals except the ad lib group was administered daily at approximately 4:00 p.m. and removed the next morning at 9:00 a.m.; animals were given water between 9:00 a.m. and 4:00 p.m. Mean consumption of ethanol/day was 12.8±0.43 g/kg on day 5, 10.8±1.19 g/kg on day 12 and 9.78±0.94 g/kg on day 19. A pair-fed chow group was not included in Part II.

After the 19 day treatment, the development of behavioral tolerance was assessed by measuring blood alcohol levels (BAL) at the time of regaining the aerial righting reflex. Rats were injected with ethanol (2.25 g/kg IP) as a 20% (w/v) solution in saline, and the aerial righting reflex was measured by dropping the rats upside down onto a foam pad from a height of 30 cm. After animals successfully landed on all four feet two consecutive times, two 40 μl samples of blood were taken from the retrorbital sinus. BAL was determined enzymatically using alcohol dehydrogenase [1].

Immediately after the behavioral testing rats were killed by decapitation, and the striatum and cerebellum were removed. The tissues were homogenized (Brinkman Polytron) in 2 mM Tris-HCl (pH 7.5) containing 2 mM EGTA and were stored at −70°C. The frozen tissues were later thawed at 37°C and were centrifuged at 20,000×g for 15 minutes at 4°C. The resulting pellets were resuspended in the Tris/EGTA buffer and were assayed for adenylate cyclase activity.

Adenylate cyclase activity was determined by measuring the conversion of α-[^{32}P]-ATP (New England Nuclear) to [^{32}P]-cyclic AMP. Reactions were carried out in a final volume of 200 μl containing 50 mM Tris-HCl (pH 7.5), 5 mM cyclic AMP, 2 mM $MgCl_2$, 1 mM theophylline, 50 μM GTP, 0.1 mM ATP (approximately 1 million cpm α-[^{32}P]-ATP), 0.1 mg/ml creatine kinase, 10 mM creatine phosphate and appropriate drugs. Reactions were initiated by the addition of tissue (36–68 μg striatal protein or 19–73 μg cerebellar protein) and were terminated after 10 minutes at 30°C with 100 μl of 50 mM Tris-HCl (pH 7.5) containing 5 mM ATP and 10% sodium dodecyl sulfate. After incubating the tubes for 15 minutes in boiling water, 20,000 cpm of [^{3}H]-cyclic AMP was added to quantify recovery, and the sample volume was increased to 1 ml with water. A modification [23] of the method of Salomon *et al.* [27] was used to isolate the [^{32}P]-cyclic AMP. Protein content was determined by the method of Lowry *et al.* [15] using bovine serum albumin (Fraction V) as a standard.

Data were analyzed by an F-test using the error mean square of the ANOVA of the 3 (Part II) or 4 (Part I) groups [30]. The *a priori* comparisons involved a determination of the effects of the ethanol treatment (ethanol-treated vs. pair-fed liquid diet), the effects of the liquid diet (pair-fed liquid diet vs. pair-fed chow) and the effects of the reduced food intake (pair-fed chow vs. ad lib).

RESULTS

Part I

Rats chronically treated with ethanol regained their aerial righting reflex at a significantly higher BAL than did rats in the pair-fed liquid diet group (Table 1). No significant dif-

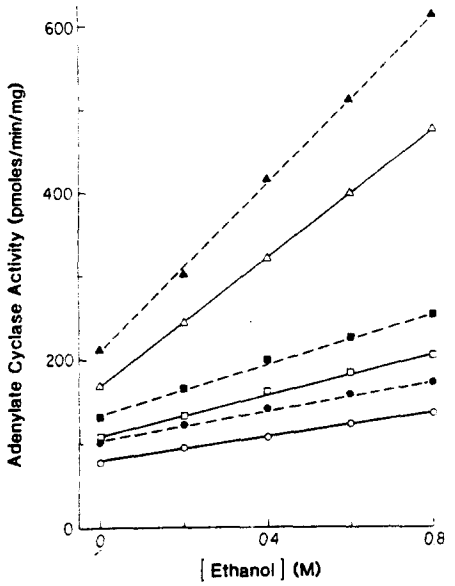

FIG. 2. Effects of chronic ethanol treatment on the sensitivity of striatal adenylate cyclase activity to ethanol *in vitro*. Rats (initial body weight 173–195 g) were treated as described in the Method section. GTP (50 μM; ○,●)-, dopamine (50 μM plus 50 μMGTP; □,■)-, and NaF (10 mM; △,▲)-stimulated adenylate cyclase activities were determined in the presence of five *in vitro* concentrations of ethanol (0–0.8 M). Data are plotted as mean of the 6 ethanol-treated animals (filled symbols) or of the 7 control animals pair-fed the liquid diet without ethanol (open symbols): S.E.M. were less than 11% of the mean.

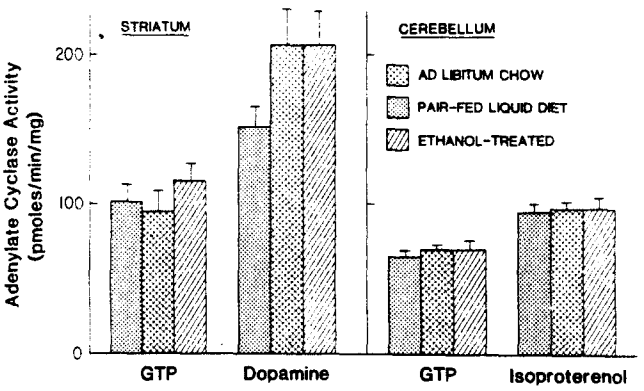

FIG. 3. Effects of chronic ethanol treatment on striatal (left) and cerebellar (right) adenylate cyclase activities. Adenylate cyclase activity in rats (initial body weight 280–385 g) chronically treated with ethanol (striped bars; N=12 for striatal tissue and N=10 for cerebellum), pair-fed the liquid diet without ethanol (stippled bars; N=9 for striatum and N=10 for cerebellum), or fed lab chow ad lib (cross-hatched bars; N=8 for striatum and N=6 for cerebellum) was determined in the presence of 50 μM GTP, 50 μM dopamine plus 50 μM GTP, or 50 μM isoproterenol plus 50 μM GTP. Data are expressed as mean±S.E.M.

ference was observed in the BAL at the time of regaining the aerial righting reflex between the two pair-fed groups or between the pair-fed chow and ad lib groups. All animals gained weight over the 19 day treatment, and as expected the pair-fed animals gained less weight than the ad lib group. The ethanol-treated group also gained less weight than the pair-fed liquid diet group. Membrane protein content per striatum, however, was not significantly different among any of the groups (data not shown). Thus, adenylate cyclase activity could be normalized on the basis of protein without biassing the results.

After the chronic ethanol treatment, basal (i.e., activity in the presence of 50 μM GTP) and dopamine-stimulated adenylate cyclase activities were significantly elevated compared to rats pair-fed with liquid diet (Fig. 1). No difference in the percent stimulation by dopamine, however, was observed between the ethanol-treated rats and animals pair-fed the liquid diet. Enzyme activity in the presence of NaF, which increases enzyme activity through an activation of the regulatory subunit of adenylate cyclase [20], was also increased. No difference in enzyme activity, however, was observed in the presence of $MnCl_2$, which directly activates the catalytic subunit of the enzyme [31]. No difference in adenylate cyclase activity was observed between the pair-fed groups or between rats fed lab chow ad lib and those pair fed with lab chow.

As previously observed [22,23] the *in vitro* addition of ethanol to striatal tissue elicits a linear and dose-dependent increase in adenylate cyclase activity (Fig. 2). The change in enzyme activity per unit concentration of ethanol (i.e., slope of ethanol dose-response plot) can therefore be used as an index of the *in vitro* sensitivity of adenylate cyclase to ethanol [23]. Chronic ethanol administration caused a parallel shift in the *in vitro* ethanol dose-response plots in the presence of GTP, dopamine plus GTP or NaF. This shift was due to the difference in intrinsic adenylate cyclase activity. When the data were expressed as the percent stimulation by *in vitro* ethanol to negate the differences in enzyme activity observed in the absence of *in vitro* ethanol, the ethanol dose-response plots were identical. No difference in the sensitivity of adenylate cyclase to ethanol *in vitro* was observed between the two pair-fed groups or between rats pair-fed chow and those fed lab chow ad lib (data not shown).

Part II

Since the development of tolerance to the inhibitory effects of *in vitro* ethanol on Na-K-ATPase activity differed amongst various brain regions [25], the effects of chronic ethanol administration on cerebellar adenylate cyclase activity were also determined. In this study the pair-fed chow group was omitted since no difference between the two pair-fed groups was previously observed. The ethanol-treated and pair-fed liquid diet groups gained a comparable amount of weight (12±1.5% and 13±1.5%, respectively), but the ad lib group still gained twice as much weight. The 19 day treatment with ethanol again resulted in behavioral tolerance; BAL at the time of regaining the aerial righting reflex was 162±24.4 mg% for the pair-fed liquid diet group and 263±24.4 mg% for the ethanol-treated animals ($p<0.001$). BAL for the rats fed lab chow ad lib was 203±4.0 mg%. Chronic ethanol administration had no effect on basal adenylate cyclase activity in cerebellar tissue or on enzyme activity in the presence of a maximally effective concentration of isoproterenol (Fig. 3). Contrary to the increase in striatal adenylate cyclase activity observed in the smaller rats (Fig. 1), with the larger rats no difference in striatal adenylate cyclase activity was found between the ethanol-treated and pair-fed liquid diet groups or between the pair-fed liquid diet and ad lib animals (Fig. 3). The sensitivity of

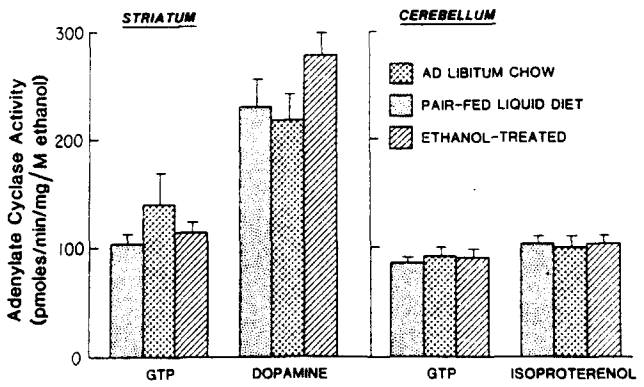

FIG. 4. Effects of chronic ethanol administration on the sensitivity of striatal (left) and cerebellar (right) adenylate cyclase activities to ethanol *in vitro*. Adenylate cyclase activity in rats (initial body weight 280–385 g) chronically treated with ethanol (striped bars; N=12 for striatum and N=10 for cerebellum), pair-fed the liquid diet without ethanol (stippled bars; N=9 for striatum and N=10 for cerebellum), or fed lab chow ad lib (cross-hatched bars; N=8 for striatum and N=6 for cerebellum) was determined in the presence of five *in vitro* concentrations of ethanol (0–0.8 M). GTP (50 μM) alone or with either 50 μM dopamine or 50 μM isoproterenol was included in all tubes. Data are expressed as the slope of the in vitro ethanol dose-response plot and are plotted as mean±S.E.M.

cerebellar and striatal adenylate cyclase to *in vitro* ethanol was also not altered by chronic alcohol administration (Fig. 4).

DISCUSSION

Although the *in vitro* addition of ethanol increases adenylate cyclase activity [6, 16, 22, 23, 32], chronic administration of ethanol did not alter the slopes of the *in vitro* ethanol dose-response plots. This indicates the sensitivity of striatal and cerebellar adenylate cyclase to *in vitro* ethanol was not changed by the chronic ethanol exposure. It is unlikely the treatment protocol used in the present study, which resulted in behavioral tolerance, would have masked an effect of the chronic treatment since no difference in the sensitivity of adenylate cyclase to *in vitro* ethanol was observed between the pair-fed groups or between the ad lib and pair-fed chow rats. The present results extend previously reported findings with rat cortical tissue [35], but conflict with the previously reported reduction in the sensitivity of basal adenylate cyclase activity in mouse striatum [10]. The reason for this discrepancy is unclear, but may be related to the different protocols used to administer the ethanol or the species used.

The present data indicate that adaptation to ethanol-induced activation of adenylate cyclase does not occur after chronic ethanol treatment. The absence of a compensatory adaptation is unusual since the potency of ethanol to alter activity of other membrane-bound enzymes is reduced after chronic treatment [8, 11, 13, 14, 25]. It is interesting to note that a basic difference also exists in the effects of *in vitro* ethanol; adenylate cyclase activity is increased by ethanol whereas Na-K ATPase activity, phospholipase A_2 activity and calcium uptake are all inhibited.

The development of tolerance to the inhibitory action of ethanol plus norepinephrine on Na-K ATPase activity varies amongst the different brain regions [25]. These workers found a direct correlation between initial sensitivity and the degree to which tolerance develops. The findings on Na-K ATPase activity [25] in conjunction with the data on adenylate cyclase activity ([35], present study) indicate that even within a particular brain region different membrane-bound enzyme systems are affected differently by chronic ethanol exposure. Although the development of tolerance involves an adaptative change in the plasma membrane [2, 7, 9, 17], this change does not appear to be a generalized membrane phenomenon. The plasma membrane is a heterogeneous structure containing both lipid domains and a nonhomogenous distribution of membrane-bound proteins [12, 28, 36]. The differential response of adenylate cyclase and Na-K ATPase to chronic ethanol treatment suggests only specific membrane domains may be altered. Whether chronic ethanol administration alters the lipid bilayer or the membrane-bound enzymes is unclear. The data does suggest that adenylate cyclase and the surrounding microenvironment are conserved during chronic ethanol administration.

In the present study with the smaller rats (initial body weight 173–195 g) chronic ethanol administration significantly increased striatal adenylate cyclase activity in the absence of *in vitro* ethanol. However, when slightly larger rats (initial body weight 280–385 g) were used, chronic ethanol exposure did not alter intrinsic enzyme activity. The reason for this discrepancy is unclear. Since the tissues for both studies were handled in an identical manner the discrepant findings were not due to methodological differences. In addition since adenylate cyclase activity was similar in the pair-fed rats and animals fed lab chow ad lib, the differing effect of the chronic treatment on intrinsic enzyme activity was not due to a difference in caloric intake. The only apparent differences between the two sets of experiments was the 1–2 month age difference of the animals and the different rates of growth. Both groups of animals are sexually competent, but neither group has reached full maturity [4]. The age difference between the two sets of rats is slight, but the two groups are at different stages of growth and maturation and, therefore, are experiencing different hormonal environments. Although speculative, the most likely explanation for the discrepant findings on intrinsic adenylate cyclase activity involves a complex interaction between the ethanol exposure and maturation of the animals. The validity of this hypothesis and elaboration of the possible interactions must, however, await further study. The data does emphasize the hazards of mechanistic interpretation of changes observed after chronic ethanol treatment.

In conclusion, the present study indicates chronic ethanol administration to rats does not alter the sensitivity of adenylate cyclase to ethanol *in vitro*. The data also indicates that the effects of ethanol exposure on intrinsic adenylate cyclase activity may not be a simple, direct effect of the chronic ethanol treatment.

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