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Genetic Differences in the Establishment of Ethanol as a Reinforcer

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RITZ, M C , F R GEORGE, C M DEFIEBRE AND R A MEISCH *Genetic differences in the establishment of ethanol as a reinforcer* PHARMACOL BIOCHEM BEHAV 24(4) 1089–1094, 1986 —Ethanol, self-administered orally, has been shown to serve as an effective reinforcer in several species Self-administration studies have also illustrated that ethanol-drinking behavior can be conceptualized as a specific type of operant behavior The use of inbred and selectively bred animals in other areas of alcohol research has provided valuable information about the contribution of genetic factors to ethanol-related behaviors Our research was designed to study genetic differences in oral self-administration in the ALKO AA (Alcohol Accepting) and ANA (Alcohol Non-Accepting) rat lines, selected for ethanol preference Thus, we applied a behavior genetic analysis to aid in determining the contribution of genetic factors to behavior, specifically drug-seeking behavior The results of our experiments indicate that genetic differences are important factors contributing to the establishment of a drug as a reinforcer At least in the case of ethanol, the drug did not act as a reinforcer in non-preferring animals Conversely, in preferring animals, ethanol was readily established as a reinforcer

Ethanol self-administration Animal models Alcohol ALKO AA and ANA rats Preference Genetic differences Behavior genetics

ETHANOL, self-administered orally, has been shown to serve as an effective reinforcer in mice, rats, rhesus monkeys, and baboons [5, 12, 24, 28]. Usually, there are two problems in establishing ethanol as a reinforcer when it is taken orally These are the aversive taste of ethanol concentrations above 6% (wt /vol.), and the delay between drinking ethanol and the onset of the interoceptive effects that follow absorption [27]. To overcome these difficulties, training procedures are used which establish ethanol as a reinforcer [21,24]. After training, animals will drink intoxicating amounts of ethanol in concentrations up to and including 32% (w/v) in preference to water [11,23] Recent research in our laboratory has shown that it is possible to train a relatively large group of rats with identical treatment histories over time, all of which self-administer sufficient ethanol within a one hour session to produce blood ethanol levels well in excess of 100 mg% [10]. Importantly, self-administration techniques have also been successfully used to establish other orally delivered drugs as reinforcers for rats and rhesus monkeys [2, 25, 26]

The use of inbred and selectively bred animals in other areas of alcohol research has provided valuable information about the contribution of genetic factors to ethanol-related behaviors, including drinking patterns such as preference [19] A common example is that C57BL/6 mice prefer ethanol whereas DBA/2 mice are strong ethanol-avoiders [4] These ideas provided the rationale for selectively breeding animals for ethanol related phenotypes. Several breeding programs to select for high and low ethanol intake have been successfully conducted in different laboratories [6, 14, 31]. Lines have also been selectively bred for high and low sensitivity to ethanol administered as an acute injection [1, 9, 18]. These studies provide not only convincing evidence of the importance of genetic factors in response to ethanol but also provide extremely valuable research tools to investigators interested in biological substrates of ethanol related behaviors

The AA (Alcohol Accepting) and ANA (Alcohol Non-Accepting) rat lines were selected from an original foundation stock using a two bottle choice paradigm. The selection criterion included the daily ratio between total ethanol consumed to total liquid intake (E/T) in combination with a de-

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termination of the ethanol consumed per unit body weight A large line difference in preference appeared quickly during the initial development of the lines, and this difference has persisted through a revitalization program conducted to protect against loss of viability associated with inbreeding The success of this selection process indicates that ethanol consumption is strongly influenced by genetic factors [6, 7, 8, 13] The line designated AA consumes almost as much ethanol as it can metabolize in a free choice situation In contrast, the ANA animals drink very small amounts under the same conditions Studies utilizing these animals have indicated line differences in acute sensitivity to doses of ethanol producing motor impairment and hypnotic effects [13, 21, 29] Little information concerning self-administration in an operant situation is available for these animals Initial evidence, however, suggests that the AA rats, previously exposed to ethanol, quickly learn to lever press for deliveries of ethanol solutions, and will work actively for the drug [30] ANA rats having the same previous experience do not respond readily for ethanol in an operant chamber Using another pair of lines selected for ethanol preference, Waller *et al* [32] illustrated that preferring rats, previously exposed to 10% (v/v) ethanol in a choice situation, will work in an operant situation to obtain intragastric infusions of ethanol while non-preferring rats will not initiate or maintain responding under identical conditions

The purpose of the present research was to systematically investigate the relationship between genetic factors regulating preference for ethanol in the AA and ANA rats and the conditions under which ethanol comes to serve as a positive reinforcer Specifically, initial tendencies to self-administer ethanol under food-satiated conditions, levels of water or ethanol intake under food-deprived conditions, and the capacity for ethanol to act as a reinforcer were studied The relationship between oral ethanol self-administration and resultant blood ethanol concentrations was also analyzed

GENERAL METHOD

Animals

Sixteen adult male rats, four months old and weighing approximately 270 g at the start of their training, were used Eight each of the Alcohol Accepting (AA) and Alcohol Non-Accepting (ANA) lines were obtained from ALKO laboratories in Finland These animals were experimentally naive, housed individually in a temperature controlled room (26°C) with a 12-hr light-dark cycle (0700–1900 lights on), and given free access to Purina rat chow and tap water prior to initiation of the experiments

Apparatus

Eight sound attenuated operant conditioning chambers (LVE 1414) were equipped with two levers and a solenoid driven liquid dipper (LVE 1351) A house light was provided for general illumination Three colored lights above each of two levers provided visual stimuli during test sessions The boxes were programmed so that lever presses on only one side resulted in dipper presentations Presses on the second lever were recorded and served as a measure of nonspecific responding but had no programmed consequence. The dipper cup (0.11 ml) remained available unless driven by a lever press at which time it momentarily dropped into a liquid-filled reservoir in order to refill with liquid Programming and data recording were controlled by equipment located in an adjacent room

TABLE 1

ORAL SELF-ADMINISTRATION OF ETHANOL IN DRUG NAIVE, FOOD SATIATED AA (N=8) AND ANA (N=8) MALE RATS

	Genotype			
	AA		ANA	
% Ethanol (w/v)	Deliveries	ml/g BW	Deliveries	ml/g BW
0	8 3 ± 1 9	0 009	4 9 ± 1 0	0 008
2	4 8 ± 0 9	0 009	2 3 ± 0 6	0 008
4	4 9 ± 0 9	0 011	2 1 ± 0 5	0 009
8	4 3 ± 1 2	0 012	1 3 ± 0 4	0 010
Retest 0	6 5 ± 0 8	0 009	1 3 ± 0 4	0 007

Values expressed as Mean ± SEM Repeated measures analysis of variance showed no significant differences BW=body weight Deliveries=number of dipper presentations

Blood Ethanol Assay

On the last day of each treatment condition duplicate 10 μl blood samples were taken from the tail of each rat immediately following the completion of the experimental session Since most ethanol drinking occurred at the start of the one hour sessions, these tail blood samples permit a conservative estimate of circulating ethanol levels We used an enzymatic assay based on the conversion of NAD to NADH during the oxidation of ethanol to acetaldehyde by the enzyme alcohol dehydrogenase. The blood samples were placed in 190 μl cold 0 55 M perchlorate, shaken, then centrifuged at 700 × g for five min One hundred seventy μl of supernatant was removed and placed in a separate test tube to which 30 μl deionized water and 200 μl 0 222 M K_2CO_3 were added The tubes were vortexed and centrifuged for two min Aliquots of 80 μl were removed in replicate and added to 640 μl cold 0 50 M Tris buffer at pH 8 8 Forty μl of 50 mM NAD^+ were next added, followed by 40 μl ADH (Sigma, 500 units/ml) The samples were vortexed and then incubated at room temperature for at least one hour, then analyzed for the formation of NADH by measuring spectrophotometric absorbance at 340 nm Samples were compared to standards which were made and analyzed concurrently with the samples

Data Analysis

Since the rats had identical treatment histories, a group design using within subjects repeated measures analysis of variance (ANOVA) was appropriate Self-administration of ethanol was measured in several ways, including lever presses, ethanol deliveries, volume of liquid consumed and blood ethanol levels

EXPERIMENT 1 DETERMINATION OF INITIAL SELF-ADMINISTRATION OF ETHANOL UNDER CONDITIONS OF FOOD-SATIATION

This experiment was designed to test whether the AA and ANA lines differ in their predisposition to self-administer ethanol There existed the possibility that the AA animals were naturally predisposed to consume ethanol and would not require training to establish ethanol as a reinforcer If the phenotype used in breeding these animals for preference

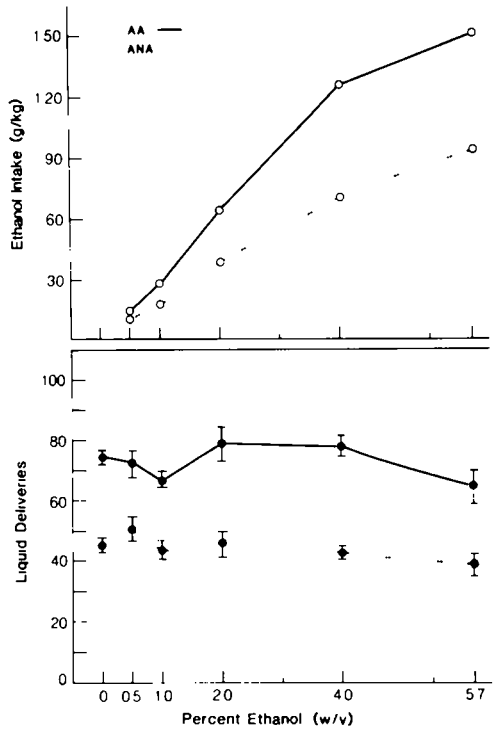

FIG 1 Concentration-response curves for ethanol self-administration by the AA (N=8) and ANA (N=8) rats under food-induced conditions on a FR 1 schedule of reinforcement Each data point represents a group mean for five consecutive test days Repeated measures analysis of variance (ANOVA) within each line, volumes of ethanol solutions consumed did not differ significantly from volumes of water consumed Upper portion shows ethanol intake (g/kg) as a function of concentration Mean BEC at 5 7% for the AA rats was 176±20 mg% (mean±SEM) and for the ANA rats was 116±24 mg%

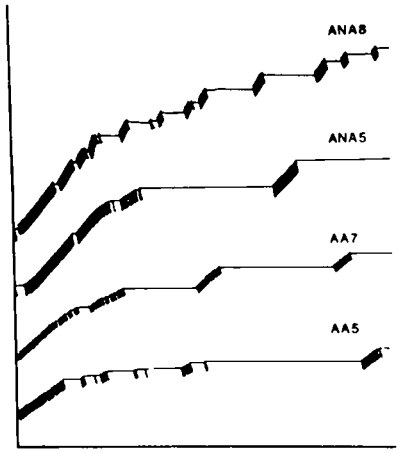

FIG 2 Cumulative records of typical animals in both lines during food-induced drinking phase of the experiment Most drug deliveries were obtained at the beginning of the session, as shown by the negatively accelerating response rates Thereafter, short bursts of responses occurred later in the session

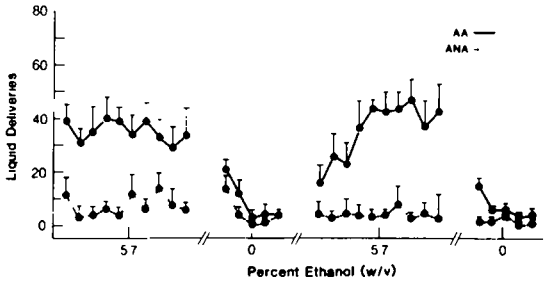

FIG 3 Liquid deliveries obtained by AA (N=8) and ANA (N=8) rats on a FR 1 schedule of reinforcement as a function of ethanol concentration on consecutive test days Repeated measures ANOVA Line, $F(1,14)=15\ 42$, $p<0\ 002$, Concentration, $F(3,42)=18\ 40$, $p<0\ 0001$, ANA, $F(3,21)=1\ 08$, n s , AA, $F(3,21)=18\ 14$, $p<0\ 0001$ AA Dunnett's t ($df=12$) 5 7% vs retest=1 71, n s , 0% vs retest=0 16, n s , 5 7% vs 0%=4 15, $p<0\ 01$, one-tailed

contained a significant taste or olfactory component, then ethanol drinking might occur in at least the AA line without special training.

METHOD

Procedure

One hour operant sessions were run at regular starting times seven days per week Initially, the experimentally naive rats were food satiated but deprived of water in their home cages All training and testing sessions were run on a FR 1 schedule, and each lever press resulted in the presentation of a dipper cup containing 0.11 ml water. Following the establishment of lever pressing, the rats were allowed free access to water in their home cages Ethanol solutions of 0% (water), 2% (w/v), 4%, and 8% were available during the sessions for 5 test days each. A 0% retest was also performed during 5 final test sessions. The rats were run daily in two groups of eight Chamber and time of day were controlled for by running four animals from each line during each of two successive one hour sessions

RESULTS

Table 1 indicates that neither AA nor ANA animals drank significant volumes of water or ethanol, and the two lines did not differ from each other. The data suggest that selection for ethanol preference did not establish a tendency in naive AA animals to drink ethanol in the operant test chamber

EXPERIMENT 2 ESTABLISHING ETHANOL AS A REINFORCER UNDER CONDITIONS OF FOOD-DEPRIVIATION

In this experiment, the rats were reduced to 75% of their free-feeding weights. The rats were induced to drink ethanol solutions by giving them their daily allowance of food prior to the operant session This strategy has been successfully used in previous studies [8]

METHOD

Procedure

During this phase of the study, rats initially received their daily food allowance in their home cages 60 minutes prior to

the start of the 1 hr test Water bottles were removed from the home cage at the time of feeding In each of ten daily sessions, the rats then lever pressed to recieve a delivery of water Water bottles were reattached to the home cages following the test sessions Once a stable pattern of water reinforced responding was established as determined by slope analysis, 0 5%, 1 0%, 2 0%, 4 0%, and 5 7% solutions of ethanol in ascending order replaced the water Each concentration was available for five consecutive sessions These concentrations of drug solution were chosen so that there would be a gradual adaptation to ethanol in order to minimize the possibility that aversions to ethanol would develop This procedure is conservative but preliminary results indicated that this type of approach was necessary to ensure that non-preferring animals such as the ANAs had sufficient exposure to ethanol

RESULTS

Figure 1 illustrates that both AA and ANA rat lines can be induced to drink pharmacologically significant amounts of ethanol Within each line no significant differences were seen between volumes of ethanol solutions consumed and water consumed Thus, as the concentration of ethanol was increased, all animals consumed more total ethanol (mg/kg body weight) Blood ethanol concentrations (BEC) indicate that animals from both lines had ingested significant amounts of ethanol Mean BEC at 5 7% for the AA rats was 176±20 mg% (mean±SEM) and for the ANA rats was 116±24 mg% Across all conditions, AA rats drank more liquid than ANA rats

Significantly, the two lines exhibited similar patterns of within session drinking during the conditions of access to ethanol Figure 2 shows the cumulative records of typical animals in both lines during this phase of the experiment Most drug deliveries were obtained at the beginning of the session, as shown by the negatively accelerating response rates Thereafter, short bursts of responses occurred later in the session

EXPERIMENT 3 TESTING FOR ESTABLISHMENT OF ETHANOL AS A REINFORCER

Ethanol reinforced responding usually persists in the absence of food induced drinking [22] The purpose of this phase of the study was to determine whether ethanol had been established as a reinforcer in the AA and ANA rats

METHOD

Procedure

The rats were now given decreasing proportions of their daily food allowance 60 minutes before the operant session in which they had access to a 5 7% solution of ethanol The rats were receiving an average of 10 grams of food daily before this phase of the experiment They were now given 4, 2, and 0 grams of food before the daily session for five successive days each Rats still had free post-session access to water and the remainder of their food allowance 15 minutes post-session in their home cages After this phase, the rats received all of their daily food allowance after the one hour session was finished To test for the establishment of ethanol as a reinforcer, the rats had access to 5 7% ethanol for 10 sessions, 0% ethanol (i e , water) for 5 sessions, 5 7% ethanol retest for 10 sessions, and 0% ethanol retest for a final 5 sessions

RESULTS

As seen in Fig 3, significant differences between lines were observed in the reward efficacy of ethanol Since the number of ethanol deliveries for the AA rats was much greater than the number of water deliveries, ethanol served as a reinforcer for the AA rats The AA's response rate fell to a level not significantly different from the ANA's response rate when tap water was substituted for ethanol during the daily sessions The AA's response rate subsequently returned to previous levels when 5 7% ethanol was again available, and again decreased significantly during the final 0% retest In contrast, ethanol did not function as a reinforcer in the ANA rats Blood ethanol levels obtained during the 5 7% retest showed large differences in ethanol intake between lines Mean BEC for the AA rats was 51±9 mg%, and 10±7 mg% for the ANA rats Some individual differences were seen in both lines One AA (AA3) responded significantly less than other AA rats One ANA (ANA8) tended to respond more than other non-preferring animals, accounting for nearly all of the ANA BEC value, though his behavior was not consistent These two rats may reflect greater heterogeneity at certain ethanol-related loci which have already been fixed by the selection process in their counterparts Significantly, six of the eight ANA rats showed no blood ethanol

GENERAL DISCUSSION

Several possibilities existed concerning the influence of genetic selection for ethanol preference on ethanol self-administration in the ALKO rats One was that ethanol would be equally well established as a reinforcer in both lines. A second possibility was that ethanol would serve as a reinforcer in both lines, but to differing degrees A third possibility was that establishment of ethanol as a reinforcer would occur in one line but not the other Finally, it was possible that neither line would self-administer ethanol under operant conditions The findings of the present study provide evidence in support of the third hypothesis Ethanol was established as a reinforcer in the AA rats but not in the ANA rats This finding complements earlier data obtained with preference procedures and extends the range of conditions over which ethanol intake exceeds that of ANA rats

In the first experiment, under conditions of food and water satiation, the AAs and ANAs did not drink significant levels of either water or ethanol even though they had been trained to lever press and drink from the dipper cups This suggests that the genetic selection of these animals probably did not involve the taste or smell of ethanol to a degree such that the rats, in particular the AAs, would drink ethanol in significant amounts without further training Thus, some training procedures can be assumed to be necessary to establish ethanol reinforcement in animals selected for preference, just as with other previously studied genetically heterogeneous animals

The results of the second experiment show that under conditions of food-deprivation it was possible to induce postprandial water and ethanol drinking in both lines During this experiment, responding within each line did not significantly change as the concentration of ethanol was increased from 0% to 5 7% As a consequence the amount of ethanol consumed increased in parallel with the changes in ethanol concentration, as shown by the blood ethanol levels seen in both lines Although not quantified, some ataxia was evident, and the amounts of ethanol consumed are known from

other studies to be pharmacologically active and to affect behavior [33]

Significantly, it was possible to induce ethanol drinking in the ANA rats These animals drank equal volumes of ethanol solutions and water postprandially, suggesting that ethanol is not aversive to the ANAs due to the drug's taste or smell. However, the data show that ANA rats consistently drank smaller volumes under all of the experimental conditions relative to the AA rats. Thus, one might conclude that ethanol came to serve as a reinforcer for the AA rats and not the ANA rats simply because the ANA rats did not drink sufficient ethanol during training. However, the ANA rats did consume volumes of ethanol high enough to produce interoceptive effects following absorption, as shown by their blood ethanol levels In addition, the history of these lines indicates that daily liquid intake is not correlated with ethanol intake. Originally, the AA line had developed both lower body weights and a higher caloric energy requirement per unit body weight than had the ANA line Consequently, the selection criteria were changed with the result that the strain difference in basic metabolic processes disappeared, while differences in body weight were reversed The pattern of metabolic and body weight differences in the ALKO lines suggests that these were artifacts caused by selection pressure on body weight without relevance to alcohol drinking [9] In fact, large line differences in preference have persisted throughout the selection process, even during the reversal of metabolic requirements for the AA and ANA lines, indicating that baseline food and water intake is not intrinsically related to preference or non-preference for ethanol

In the third experiment when drinking was no longer induced by eating, the ANA rats drank only small volumes of water and ethanol solutions. In contrast, the AA rats drank significantly more ethanol than water. Under these experimental conditions, the AAs did not differ from the ANAs in water intake These findings are consistent with ethanol serving as a reinforcer only for the AA rats. The ANA rats are apparently neither adverse to nor reinforced by the effects of ethanol, a result which is important in view of consistent findings indicating that food deprivation increases intake of ethanol and other drugs [3,20] Our results suggest that food deprivation may enhance drug intake only in those animals genetically predisposed to accept a particular drug as a reinforcer The relevance of these findings is that the increase in ethanol intake during food deprivation can no longer be attributed simply to caloric factors, since ANA animals did not consume ethanol under conditions of food deprivation in a manner similar to the AA rats, which experienced identical treatment histories

Previous self-administration experiments utilizing genetically uncontrolled subjects suggest that ethanol should have been established as a reinforcer for both the AA and ANA rats Therefore, the results of our experiments indicate that, at least under the environmental constraints outlined in this study, genotype is a critical factor in determining the reinforcing efficacy of ethanol Further, this study demonstrates the experimental control possible with the use of genetically defined animals, even when complex operant behaviors are being measured. The results described here, along with those in a recent paper by Waller and coworkers [32], indicate that genetic analyses provide useful information in operant conditioning studies of ethanol reinforced behavior

The study and comparison of ethanol intake in operant situations using animals genetically selected for differences in ethanol preference should serve to integrate findings from preference studies with findings from the more general area of drug reinforced behavior. In preference studies, rats and inbred strains of mice have been found to manifest, respectively, large between-individual and between-strain variations in ethanol preference For example, the mean preference ratio (10% ethanol/water) and intake (g/kg/day) for the heterogeneous N/Nih male stock are 1.23 and 1.4, respectively. These values are approximately midway among those of eight inbred rat strains [15]. In another study, the outbred Wistar rats were shown to consume less than 3.5 g/kg ethanol/day, but a small percentage of these animals consumed 6–8 g/kg per day [16]. This large variability in preference served as the selection basis for the ALKO AA and ANA lines as well as the P and NP rat lines. The outcome of these selection programs is important. Ethanol intake (g/kg/day) for AA and P males is 5 8±1.8 [13] and 6.3±1 7 [16], respectively, while intake for the ANA and NP males is 1.8±1 2 [13] and 1.0±0.7 [16], respectively Such information suggests that selective breeding has produced lines of rats in which ethanol drinking behavior, as described by the preference model, is maximized in one line and minimized in the other

It would therefore be expected that AA or P rats might also consume significantly greater quantities of ethanol in the operant situation. However, self-administration has been studied in Sprague-Dawley and Wistar rats under conditions similar to those used in the current experiments. The mean ethanol intake when 8% ethanol was available during one-hour sessions was 0.94 g/kg and 1.16 g/kg, respectively [21]. Though one must use caution in making direct comparisons due to differences in experimental conditions, this level of intake seems similar to that of the AA rats when 5 7% ethanol was available in the test sessions. More recently, Sprague-Dawley rats, trained in an analogous manner and tested in the same chambers in which the ALKO animals were tested, consumed 2.1 g/kg when given access to 8% ethanol in one-hour operant sessions. Thus, it seems possible that high ethanol intake in a preference test may not be completely generalizable to other tests of ethanol drinking behavior. Since the preference test is dependent only on minimal learning and work, it is possible that biological factors which are necessary for and facilitate high ethanol consumption in the operant situation, requiring the completion of learned sequences of behavior in order to receive access to drug solutions, may have been lost or at least allowed to segregate randomly within the ALKO lines. Additional studies are currently underway to investigate the relationship between ethanol preference, ethanol self-administration and their common biological substrates.

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