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SELECTIVE MOUSE BREEDING FOR SHORT ETHANOL SLEEP TIME HAS LED TO HIGH LEVELS OF HEPATIC AROMATIC HYDROCARBON (Ah) RECEPTOR

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Abstract—Following a selective breeding program of heterogeneous mice for more than 30 generations, *SS* ("short sleep") and *LS* ("long sleep") lines have been developed on the basis of their sleep times when challenged with a single intraperitoneal dose of ethanol. The aromatic hydrocarbon responsiveness (*Ah*) locus encodes the Ah receptor, which regulates the induction of certain drug-metabolizing enzymes by polycyclic aromatic compounds such as 3-methylcholanthrene and tetrachlorodibenzo-*p*-dioxin. The C57BL/6 inbred mouse strain (B6; Ah^b/Ah^b) has a high-affinity Ah receptor, while the DBA/2 inbred mouse strain (D2; Ah^d/Ah^d) has a low-affinity Ah receptor. We show here that the *SS* inbred mouse line exhibits markedly elevated hepatic levels of the high-affinity Ah receptor, while the *LS* outbred mouse line contains the low-affinity Ah receptor. Among progeny of $(B6D2)F_1 \times D2$ backcross, the *b/d* heterozygote (having the high-affinity Ah receptor) was found to be several times more resistant than the *d/d* homozygote to a single dose of intraperitoneal ethanol. The D2.B6-Ah^b congenic line is also several times more resistant to intraperitoneal ethanol than the B6.D2-Ah^d congenic line. We found that the waking blood ethanol levels are the same in *b/d* and *d/d* mice, suggesting that the relative ethanol resistance in *b/d* mice cannot be explained on the basis of a difference in central nervous system sensitivity. There are no differences between *SS* and *LS* mice or between *b/d* and *d/d* mice with regard to (i) blood acetaldehyde levels after a single intraperitoneal dose of ethanol, or (ii) hepatic alcohol dehydrogenase activities. There is a difference in the rate of ethanol elimination: *SS* more rapid than *LS*; *b/d* more rapid than *d/d*. Although *SS* mice have lower hepatic aldehyde dehydrogenase activities (cytosolic, mitochondrial low-K_m; and mitochondrial high-K_m forms) than *LS* mice, *b/d* and *d/d* do not show this difference. These data suggest that a selected mouse breeding program, based on resistance to a single intraperitoneal dose of ethanol, selects concurrently for the hepatic high-affinity Ah receptor. This selective advantage cannot be explained on the basis of changes in alcohol dehydrogenase or aldehyde dehydrogenase activities and might provide insight into the nature of the endogenous ligand for the Ah receptor.

Numerous studies have demonstrated differences among inbred mouse strains in response to, and preference for, ethanol [1, 2]. Perhaps the best evidence suggesting that initial response to ethanol is determined by genetic factors comes from the observation that selective breeding from a heterogeneous stock of mice§ has resulted in two selected lines that differ by a large factor in response to ethanol [3]. The ethanol selection process was initiated more than two decades ago with the *HS* mice.¶ Approximately 100 *HS* mice were injected intraperitoneally with ethanol (3.3 g/kg); this dose was sufficient to cause loss of the righting response (ethanol-induced "sleep") in nearly all of the animals. More than 40 mice with the shortest ethanol-induced sleep times were mated together and served as the progenitors for the short sleep (*SS*) line. Similarly, more than 40 mice with the longest ethanol-induced sleep times were mated together to generate a long sleep (*LS*) line. At present more than 40 generations have passed, each generation selected on the basis of decreasing (*SS*) or increasing (*LS*) sleep times in response to a single intraperitoneal dose of ethanol.

These two mouse lines have demonstrated their

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§ Heterogeneous stock (*HS*) mice originated from an 8-way cross-population developed at the University of California (Berkeley) by Dr Gerald E. McClearn in collaboration with Dr W. Meredith. The eight original strains [3, 4] included: A, BALB/c, C3H/2, C57BL, RIII, AKR, DBA/2, and Is/Bi; all were sublines at the Berkeley Cancer Research Genetics Laboratory at the time. The mice later were imported to the Institute of Behavioral Genetics, University of Colorado (Boulder), where they are being maintained as a base stock with 40 mated pairs in each generation. Mates are assigned randomly with the restriction that mates cannot have a common grandparent. With such a large sample of matings used in each generation, this arrangement will maximize outbreeding and prevent many genes from ever segregating. These mice are believed to be more heterogeneous than any wild mouse colony or any outbred or randombred laboratory animal.

¶ Abbreviations include: *HS*, heterogeneous stock; *SS*, "short ethanol sleep time" selected outbred mouse line; *LS*, "long ethanol sleep time" selected outbred mouse line; B6, the C57BL/6N inbred strain; D2, the DBA/2N inbred strain; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; [^{3}H]TCDD, [^{3}H-1,6]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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uniqueness as animal models for studying the actions of ethanol. For example, the two lines were shown to differ in response to ethanol, largely because of dissimilarities in central nervous system sensitivity to ethanol; waking blood ethanol is almost twice greater in *SS* than in *LS* mice [5]. A statistically significant difference between the lines has also been reported in the rate of ethanol elimination: *SS* mice clear ethanol from the body more rapidly than *LS* mice [6].

The three systems known to metabolize ethanol include (a) alcohol dehydrogenase, (b) catalase, and (c) cytochrome P450-mediated monooxygenase(s). Ohnishi and Lieber [7] first determined the existence of a catalase-free P450-mediated microsomal ethanol-oxidizing system (MEOS). Subsequently, the ethanol-inducible P450 genes have been cloned from rat, human and rabbit and shown to be members of the P450IIE subfamily [8]. Interestingly, although no differences in basal MEOS activity were found between *SS* and *LS* mice, 3-methylcholanthrene [9] as well as ethanol [10] treatment was found to enhance MEOS activity in *SS* but not in *LS* mice.

The aromatic hydrocarbon responsiveness (*Ah*) locus is known to encode the Ah receptor, which regulates the induction of mouse P_1450 and P_3450 and several other enzyme activities by 3-methylcholanthrene [reviewed in Refs 11 and 12]. In this report we show an interesting correlation between selective breeding for short ethanol sleep time and high levels of the hepatic high-affinity Ah receptor.

MATERIALS AND METHODS

Materials. [^{3}H]TCDD (52 Ci/mmol) was purchased from KOR Isotopes (Cambridge, MA). Nonlabeled TCDD was a generous gift from Dow Chemical Company (Midland, MI). Zoxazolamine (2-amino-5-chlorobenzoxazole) was generously supplied by McNeil Laboratories (Fort Washington, PA).

Animals. B6 (Ah^b/Ah^b) and D2 (Ah^d/Ah^d) mice were obtained from the Veterinary Resources Branch, National Institutes of Health (Bethesda, MD). Phenotyping of the heterozygotes (b/d) and homozygotes (d/d) from the (B6D2)F_1 × D2 backcross was carried out using the zoxazolamine paralysis test [13]. The *SS* and *LS* mice were weanling males provided by the Institute for Behavioral Genetics (University of Colorado, Boulder); these mice represented the 34th generation.

The Ah receptor assay. Mice no more than 6 weeks old were used. Preparation of the 105,000 *g* supernatant fraction from individual livers and the sucrose density gradient analysis were performed as previously detailed [14]. Briefly stated, the cytosol was treated with 10 nM [^{3}H]TCDD for 1 hr at 4° in the presence or absence of 1.0 μM nonlabeled TCDD. Dextran-charcoal adsorption was carried out to remove nonspecifically bound [^{3}H]TCDD and TCDD. Centrifugation was then performed at 235,000 *g* for 16 hr at 2° in a linear 5% to 20% sucrose density gradient, following which the radioactivity of each of 25 200-μl fractions was determined by scintillation spectrometry. The concentration of receptor was estimated in femtomoles per mg of cytosolic protein [14]. Protein determinations were performed with bovine serum albumin as the standard [15].

Ethanol-induced sleep-time test. This procedure was carried out by a standard protocol [16]. Between 9 a.m. and 10 a.m. in several independent experiments, groups of mice were timed for sleep following a single intraperitoneal dose of ethanol. After losing the righting response, the animals were placed on their backs in a V-shaped trough. The length of time between the loss of, and regaining, the righting response was recorded. Animals were judged to be "awake" when they could right themselves three times in 30 seconds. In some experiments, immediately after the mouse had regained its righting response, a 10-μl blood sample was obtained with a capillary pipette from the retroorbital sinus. This sample was immediately placed in a 12 × 75-mm test tube containing 0.99 ml of an aqueous isopropanol (25 g/100 ml H_2O) solution; the isopropanol serves as an internal standard. The tube was stoppered and placed on ice until the sample was assayed for its ethanol content.

Serum ethanol determinations. The blood samples were assayed for ethanol alone, without determining acetaldehyde levels, according to the published procedure [17]. The sealed tubes were incubated at 56° for 15 min. A 1-ml aliquot of the head-space gas was removed with a gas-tight syringe and injected into a Beckman GC-45 gas chromatograph equipped with a Porapak Q column and flame-ionization detector. The inlet temperature was maintained at 110°, while the column and detector temperatures were 150° and 190°, respectively. Helium served as the carrier gas at a flow rate of 55 ml/min. Hydrogen and air flow rates were 45 and 300 ml/min, respectively. Peak areas were determined by triangulation and the ratios of ethanol-to-isopropanol peak areas were calculated. These values were compared with a standard curve.

Acetaldehyde and ethanol determinations. In other experiments, the concentrations of acetaldehyde and ethanol were determined together by the method of Stowell [18]. Ethanol was prepared as a 35% solution, and a single intraperitoneal dose (3 g/kg) was administered. Blood was obtained from the retroorbital sinus 75 min after the ethanol injection. The blood sample (50 μl) was placed in a conical tube containing 0.95 ml of an ice-cold semicarbazide solution (16 mM NaCl, 29.9 mM NaH_2PO_4, 70.1 mM Na_2HPO_4, 6 mM semicarbazide HCl). The tubes were immediately sealed with Parafilm and spun in a cold clinical centrifuge for 10 min at maximal speed. A 700-μl aliquot of the resulting supernatant was placed in a 12 × 75 mm tube containing 0.2 ml of 3 N HCl. The tubes were stoppered and placed on ice until analysis for ethanol and acetaldehyde content.

Standard curves for both ethanol and acetaldehyde were prepared daily. The stoppered tubes were incubated for 15 min at 65°. A 2.5-ml headspace sample was removed with a gas-tight syringe and injected into a Hewlett-Packard 5710-A gas chromatograph equipped with a 3380-A integrator, a Supelco Carbopack B column and a flame-ionization detector. The inlet temperature was maintained at 120°, the

column at 86°, and the detector 320°. Gas flow rates were the same as described above. Peak areas were compared with standards.

Additional blood samples were taken 120, 165 and 210 min after ethanol treatment of *b/d* and *d/d* mice. Acetaldehyde and ethanol concentrations were determined. The rate of ethanol elimination for individual animals was measured by estimating both the slope of the disappearance curve and the area under the ethanol concentration–time curve.

Enzyme assays. The hepatic subcellular fractionation was carried out as described [19]. Alcohol dehydrogenase activity was determined in a reaction mixture consisting of 25 mM sodium phosphate and 200 mM semicarbazide buffer (pH 7.4), 16 mM ethanol, 1 mM NAD, and 50 μl of the cytosolic fraction in a final volume of 1 ml. Reactions were initiated by the addition of enzyme and were carried out at 30°. Blanks contained no substrate. The change in optical density was monitored at 340 nm. The initial rate (90 to 120 sec) was used to calculate activities. Protein was determined [15], and activity was calculated in terms of nmol of NADH formed/min/mg protein or per g liver.

Cytosolic aldehyde dehydrogenase activity was measured with 2.5 mM acetaldehyde as substrate, 1 mM NAD, 1 mM pyrazole (to inhibit alcohol dehydrogenase), 25 mM sodium phosphate buffer (pH 7.4), and 50 μl of the cytosolic fraction. Mitochondrial aldehyde dehydrogenase was determined similarly, except two acetaldehyde substrate concentrations were used: 2.5 mM and 50 μM. Blanks contained no substrate. NADH formation was monitored for the first 3 min. Activity was calculated in terms of nmol of NAD formed/min/mg protein or per g liver.

Data analysis. For the sleep-time studies, serum acetaldehyde and ethanol determinations, and liver alcohol dehydrogenase and aldehyde dehydrogenase assays, the data were initially analyzed by a one-way analysis of variance. Because no major sex effects were observed, the data from males and females were subsequently combined. Line comparisons were made using Student's *t*-test. *SS* vs *LS* or *b/d* vs *d/d* comparisons were made; no *SS-LS* vs *b/d-d/d* comparisons were sought.

RESULTS

Differences in Ah receptor between SS *and* LS *mice*

In the cross between the B6 and D2 inbred mouse strains, the induction of P_1450 and P_3450 by 3-methylcholanthrene is expressed as an autosomal dominant trait [20], whereas Ah receptor levels are inherited as an additive trait [11, 14, 21]. This means that, although the high-affinity Ah receptor levels are only half as high in *b/d* than *b/b* mice, both *b/d* and *b/b* mice exhibit similar levels of induced P_1450 and P_3450. On the other hand, hepatic P_1450 and P_3450 are not induced by 3-methylcholanthrene in the *d/d* mouse, due to the low-affinity Ah receptor that cannot be measured in the commonly used sucrose density gradient assay.

Interestingly, the *SS* outbred line was found to have very large hepatic concentrations of a high-affinity Ah receptor (Fig. 1), whereas the *LS* outbred

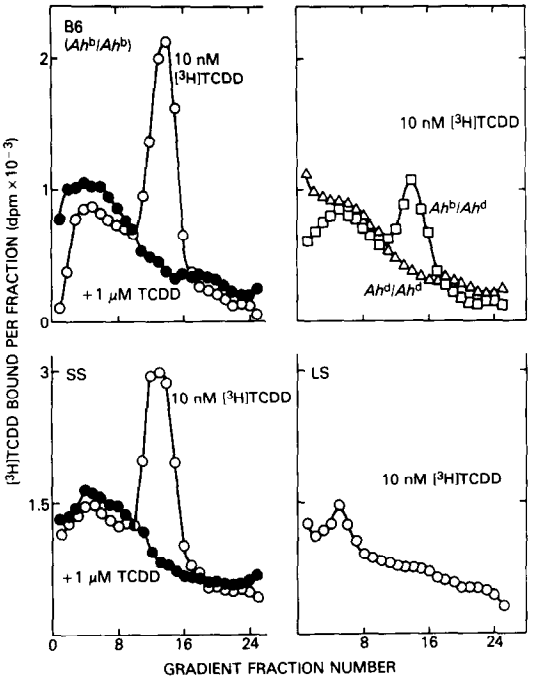

Fig. 1. Sucrose density gradient analysis of the cytosolic Ah receptor in the liver of a B6 mouse (*b/b*), upper left; individual *b/d* and *d/d* offspring of the $(B6D2)F_1 \times D2$ backcross, upper right; an *SS* mouse, lower left; and an *LS* mouse, lower right. Open symbols denote exposure of cytosol to 10 nM [^{3}H]TCDD alone; closed symbols denote exposure of cytosol to 10 nM [^{3}H]TCDD plus 1.0 μM nonlabeled TCDD. A 100-fold excess of nonlabeled TCDD completely displaces the specifically bound [^{3}H]TCDD from the Ah receptor, usually found in fractions 11 to 16. Samples with the 100-fold excess of nonlabeled TCDD are illustrated at upper and lower left. Note difference in the ordinates at top and bottom. The *b/d* heterozygote and the *d/d* homozygote had been phenotyped 12 days before this experiment by means of the zoxazolamine paralysis test [13].

line appears to have a low-affinity Ah receptor. Statistical analysis derived from individual assays on B6, D2, $(B6D2)F_1$, *b/d* and *d/d* offspring from the $(B6D2)F_1 \times D2$ backcross, *SS*, and *LS* mice is shown in Table 1. The results with *SS* and *LS* mice suggest that elevated levels of the hepatic high-affinity Ah receptor are correlated with short ethanol-induced sleep times.

Differences in acute ethanol response between b/d *and* d/d *mice*

The B6 mouse strain has long been known to be more tolerant of ethanol than the D2 mouse strain [1, 2]. The B6 and D2 inbred mouse strains differ, however, at thousands of loci. To randomize these loci and study the effect of the high-affinity Ah receptors on acute ethanol response, we examined the *b/d* and *d/d* progeny from the $(B6D2)F_1 \times D2$ backcross (Fig. 2). Those backcross progeny with the high-affinity Ah receptor were found to have significantly shorter ethanol sleep times. The *d/d* mice slept, on average, slightly more than twice as long as the high-affinity Ah receptor-containing *b/d*

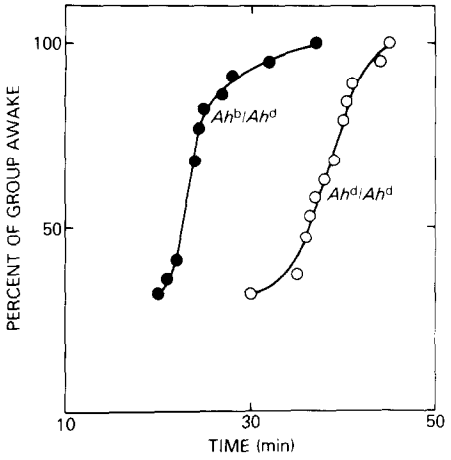

Fig. 2. Ethanol-induced sleep times of *b/d* heterozygotes (N = 22) and *d/d* homozygotes (N = 19) following an acute dose of intraperitoneal ethanol (3.7 g/kg). The *b/d* and *d/d* offspring from the (B6D2)F_1 × D2 backcross had been phenotyped 2 weeks prior to this experiment by means of the zoxazolamine paralysis test [13]. A second experiment with similarly sized groups yielded virtually identical results.

sitivity. This finding also implies that the difference in waking blood ethanol levels between *SS* and *LS* mice is an additional event that has cosegregated during the two-decade selective breeding program and is not correlated with the concentrations of hepatic high-affinity Ah receptor.

Blood acetaldehyde levels

Since the *b/d* mice exhibit a shorter ethanol sleep time than the *d/d* mice, yet show no difference in waking blood ethanol, these data suggest a dissimilarity in ethanol disposition. Following an acute dose of ethanol (Table 3), we found no difference in blood acetaldehyde, either between the *SS* and *LS* lines or between the *b/d* and *d/d* mice.

Ethanol elimination rates

The slopes of the ethanol disappearance curves for the *SS* and *LS* lines are known to be significantly different [22]. We found no apparent difference in slope (Fig. 3) between the *b/d* and *d/d* animals. One might conclude from these data that the high-affinity Ah receptor is not associated with differences in the metabolism of ethanol. When the areas under the ethanol concentration–time curves were calculated

Table 1. Cytosolic Ah receptor concentrations from the liver of several types of mice

Mouse	*Ah* phenotype*	N	Ah receptor (fmol/mg cytosolic protein)
B6	B6-like	6	57 ± 11
(B6D2)F_1	B6-like	5	32 ± 7
D2	D2-like	4	1 ± 1
b/d from backcross	B6-like	5	26 ± 15
d/d from backcross	D2-like	4	1 ± 1
SS	B6-like	10	85 ± 15
LS	D2-like	6	1 ± 2

The number (N) of individual untreated mice assayed is shown. There was no treatment of these mice with any drugs for 12 days preceding this assay. Values are expressed as mean ± SD. All mice were between 4 and 6 weeks of age and of either sex.

* Known from previous studies in this laboratory or determined by the zoxazolamine paralysis test [13].

animals (Table 2). As had already been established, the *LS* mice sleep longer than the *SS* mice, even though they must be given a substantially lower dose of ethanol in order to perform the experiment.*

Although a significant difference in waking blood ethanol levels was found between the *SS* and *LS* animals (Table 2), no difference was found for the offspring from the (B6D2)F_1 × D2 backcross. This observation suggests that the dissimilarity in sleep time observed between *b/d* and *d/d* mice might not reflect differences in central nervous system sen-

* The two lines presently differ to such an extent that no ethanol dose can be found at which both lines will sleep. The lowest effective dose for *SS* mice is fatal for the majority of *LS* mice.

Table 2. Comparison of ethanol sleep times and waking blood ethanol concentrations in the different types of mice

Mouse	N	Sleep time (min)	Waking blood ethanol (mM)
SS	10	48 ± 9.6[a]	113 ± 3.9[b]
LS	10	84 ± 3.2[a]	61 ± 2.1[b]
b/d	7	22 ± 7.4[c]	62 ± 3.7
d/d	7	50 ± 13.8[c]	62 ± 2.8

LS mice were administered 2.5 g of ethanol per kg, whereas *SS* mice received 4.1 g/kg. The *b/d* and *d/d* mice were administered a 3.7 g/kg dose. Blood ethanol content was determined at the time of regaining the righting response. Values are reported as the means ± SE. Superscripts a, b and c = significantly different ($P < 0.05$) from the similarly designated group.

Table 3. Blood acetaldehyde concentrations in the different types of mice

Mouse	N	Acetaldehyde levels (μM)
SS	20	35 ± 5.0
LS	20	37 ± 6.3
b/d	10	42 ± 7.2
d/d	10	35 ± 6.3

Animals were injected with a single ethanol dose (3 g/kg), and blood acetaldehyde content was determined by gas chromatography 75 min later. Data are reported as means ± SE.

Table 4. Ethanol elimination rates in different types of mice

Mouse	N	Negative slope (mM/hr)	Area under the ethanol concentration–time curve (mM-min)
SS	6	23 ± 0.5[a]	760 ± 12[b]
LS	6	15 ± 0.9[a]	840 ± 21[b]
b/d	10	17 ± 1.1	600 ± 60[c]
d/d	10	17 ± 1.1	760 ± 19[c]

The ethanol dose was 4.1 g/kg for each *SS* and *LS* mouse and 3.7 g/kg for each *b/d* and *d/d* mouse. Ethanol elimination was determined by taking blood samples 2, 3, 4, and 5 hr later. Values are expressed as means ± SD. Superscripts a, b and c = significantly different ($P < 0.05$) from the similarly designated group.

(Table 4), however, significant differences between the *SS* and *LS* lines and between the *b/d* and *d/d* animals were observed. In both cases, the high-affinity Ah receptor-containing groups have the smaller areas under the concentration–time curves.

Hepatic alcohol dehydrogenase activities

A smaller area under the curve may represent a more rapid elimination rate, faster ethanol metabolism, or a combination of both. Table 5 shows that the *SS* and *LS* lines do not differ in alcohol dehydrogenase activity. Similarly, the *b/d* and *d/d* mice do not differ in alcohol dehydrogenase activity. The rate of ethanol metabolism would be related to the total amount of alcohol dehydrogenase in the animal, which would be derived almost exclusively from liver. The liver weights, as a fraction of total body weight, for the *SS*, *LS*, *b/d* and *d/d* mice were determined not to be significantly different.

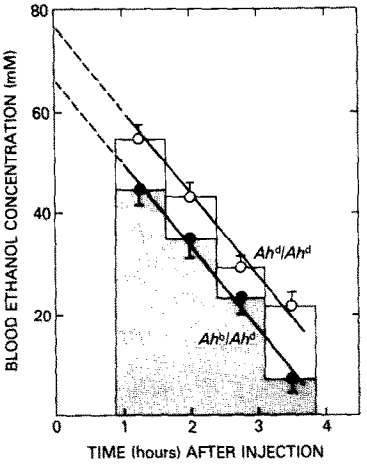

Fig. 3. Blood ethanol levels in *b/d* and *d/d* mice (N = 10 at each time point) as a function of time after an acute intraperitoneal dose of ethanol (3.7 g/kg). Areas under the concentration–time curves (shaded) were calculated by making histograms from the four points and summarizing the areas of each of the four rectangles. Similar results were obtained in a second experiment with the same number of individual *b/d* and *d/d* mice. Brackets denote standard error.

Hepatic aldehyde dehydrogenase activities

Differences between the *SS* and *LS* lines in liver aldehyde dehydrogenase activity are significant (Table 6), with the *LS* line having almost two-fold greater activity in both the cytosolic and mitochondrial fractions. No significant difference between the *b/d* and *d/d* groups was detected.

DISCUSSION

The data in this report show that a selective breeding program for short ethanol sleep time, during a period of more than two decades, has led to the selection of mice having large hepatic concentrations of the high-affinity Ah receptor. Using *b/d* and *d/d* mice from the $(B6D2)F_1 \times D2$ backcross, in which all loci that differ between B6 and D2 mice can be randomized (except alleles at the *Ah* locus), we show that the presence of the high-affinity Ah receptor is associated with a protective response against a single

Table 5. Liver alcohol dehydrogenase activities in different types of mice

Mouse	N	Specific enzyme activity (nmol/min/mg cytosolic protein)	(nmol/min/g wet weight liver)
SS	10	11 ± 0.80	790 ± 39
LS	10	9.3 ± 0.55	830 ± 40
b/d	10	13 ± 0.75	950 ± 39
d/d	10	14 ± 0.67	1,070 ± 42

Values are expressed as means ± SE.

Table 6. Liver aldehyde dehydrogenase activities in different types of mice

Mouse	Cytosolic enzyme activity (nmol/min/mg cytosolic protein)	(nmol/min/g wet weight liver)
SS	14 ± 1.6[p]	1050 ± 100[q]
LS	21 ± 1.2[p]	1930 ± 120[q]
b/d	18 ± 1.5	1410 ± 190
d/d	17 ± 1.4	1340 ± 160
	Mitochondrial low-K_m enzyme activity	
SS	2.7 ± 0.41	12 ± 1.7[r]
LS	3.2 ± 0.47	17 ± 2.3[r]
b/d	4.3 ± 0.33	23 ± 1.9
d/d	4.1 ± 0.47	28 ± 2.1
	Mitochondrial high-K_m enzyme activity	
SS	17 ± 1.0[s]	73 ± 6.9[t]
LS	23 ± 2.2[s]	125 ± 7.6[t]
b/d	26 ± 1.3	139 ± 8.0
d/d	22 ± 1.5	155 ± 10

Values are expressed as means ± SE. N in each group = 10. Superscripts p, q, r, s, t = significantly different ($P < 0.05$) from the similarly designated group.

intraperitoneal dose of ethanol. Further, we have shown that our results cannot be explained simply on the basis of differences in liver alcohol dehydrogenase or aldehyde dehydrogenase activities, as well as waking blood ethanol concentrations following the acute intraperitoneal dose.

Of the eight inbred strains originally used to generate the *HS* line [3], it is known that the A, BALB/c, C3H, C57BL and RIII strains have a high-affinity Ah receptor and that the AKR, DBA/2 and Is/Bi strains have a low-affinity Ah receptor [23]. Several allelic variants of the *Ah* response have been described phenotypically [20, 24, 25], and two allelic variants of the Ah receptor have been characterized biochemically [26]. These alleles have been named Ah^b for the C57BL/6, Ah^d for the DBA/2, Ah^h for the C3H/He and Ah^k for the AKR inbred strains [24]. The genetic repertoire of the original *HS* mice thus might be expected to have at least four distinct alleles, and possibly as many as eight different alleles, available for segregation in the *SS* and *LS* lines. Interestingly, *SS* mice contain about 50% more Ah receptor than B6 mice. Until these measurements, the B6 strain had been regarded as having the highest hepatic Ah receptor concentration of any inbred mouse strain examined.

A larger area under the ethanol concentration–time curve (Table 4) suggests that the *LS* mice, when compared with the *SS* mice, may have a decreased rate of ethanol elimination, slower ethanol metabolism, or a combination of both. Since the *LS* line has, if anything, higher aldehyde dehydrogenase activity, plus alcohol dehydrogenase activity that is not significantly different from that of the *SS* line (Tables 5 and 6), the larger area under the *LS* ethanol concentration–time curve must represent a lower rate of ethanol elimination. The same conclusions can be made about the *d/d* and *b/d* mice: the *d/d* mice appear to exhibit a decreased rate of ethanol elimination.

Factors affecting absorption of ethanol from the peritoneal cavity might contribute to the impression that *LS* mice have a lower rate of ethanol elimination. What might these factors be? A lowering of body temperature and inflammation can affect absorption. Intraperitoneal ethanol is known to produce an acute chemical peritonitis and a transient lowering of body temperature [27]; if this were to occur more extensively in the *d/d* than *b/d* mice, one might calculate an apparent larger V_D in *d/d* than *b/d* mice. In fact, although experiments looking for differences in the extent of chemical peritonitis have not been performed, *LS* mice are known to have more central nervous system prostaglandins formed than *SS* mice following a single dose of ethanol [27]. It is intriguing that benoxaprofen, a nonsteroidal anti-inflammatory agent, decreases halogenated hydrocarbon toxicity and requires the Ah receptor in the chick embryo, suggesting that the high-affinity Ah receptor might play some role in arachidonic acid metabolism or other mediation of inflammation [28]. Thus, the selection program of the *SS* line during a two-decade period may actually represent the selection of a mouse more resistant to ethanol-induced hypothermia and peritonitis. The concurrent segregation of the hepatic high-affinity Ah receptor in these mice might indicate that this receptor plays an important role in inflammation.

The endogenous ligand for the Ah receptor has not yet been identified. This study raises the possibility that some second messenger, or metabolic intermediate in the arachidonic acid pathway or other response to inflammation, might be the endogenous ligand for the high-affinity Ah receptor.

Finally, acute treatment with ethanol is known to produce a swift increase in alcohol metabolism (SIAM) in rats, mice, humans and perfused rat livers [29]. The ethanol in the SIAM experiments is usually administered intragastrically or by inhalation. Most likely, inflammation (such as chemical peritonitis)

would not occur following administration of ethanol by either of these routes. Furthermore, enhanced alcohol dehydrogenase activity is believed to be involved in the etiology of the swift increase in alcohol metabolism [29]. We therefore believe that the selective breeding program for large levels of the hepatic high-affinity Ah receptor in *SS* mice is independent from the mechanism of swift increase in alcohol metabolism.

Note added in proof—The D2.B6-*Ah*^{b} and B6.D2-*Ah*^{d} congenic inbred mouse lines have been developed in our laboratory [24]. Briefly summarized, the D2.B6-*Ah*^{b} represents an inbred line containing DBA/2N alleles in >95% of its genes, and for 40 generations this line has been selected for the presence of the C57BL/6N allele (encoding the high-affinity Ah receptor) at the *Ah* locus. Conversely, the B6.D2-*Ah*^{d} represents an inbred line containing C57BL/6N alleles in >95% of its genes, and for 29 generations this line has been selected for the presence of the DBA/2N allele (encoding the low-affinity Ah receptor) at the *Ah* locus. Use of congenic lines specifically selected for allelic differences at the *Ah* locus should help rule out the effects of ancillary genes that might play a role in the acute response to intraperitoneal ethanol. We found that the B6.D2-*Ah*^{d} mice slept, on the average, almost three times longer than the D2.B6-*Ah*^{b} mice (Fig. 4). Interestingly, large individual variations were seen in these experiments. These data strongly support our conclusion that a correlation exists between short ethanol sleep time and presence of the high-affinity Ah receptor.

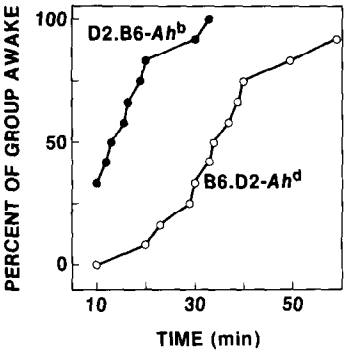

Fig. 4. Ethanol-induced sleep times of D2.B6-*Ah*^{b} and B6.D2-*Ah*^{d} congenic inbred mouse lines (N = 12 for both groups), following an intraperitoneal dose of ethanol (3.4 g/kg). Development of these congenic lines has been described [24]; the current generations used in this experiment are F_{40} and F_{29}, respectively. The last B6.D2-*Ah*^{d} mouse did not awaken until 105 min. These mice had been phenotyped 1 week prior to this experiment by use of the zoxazolamine paralysis test [13]. Two additional experiments yielded similar results.

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