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The Swift Increase in Alcohol Metabolism (SIAM) in Four Inbred Strains of Mice[1]

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THURMAN, R. G., B. U. BRADFORD AND E. GLASSMAN. *The swift increase in alcohol metabolism (SIAM) in four inbred strains of mice.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 171–175, 1983.—Ethanol metabolism increases two to three hours after the administration of ethanol. This phenomenon, called the Swift Increase in Alcohol Metabolism (SIAM), has been compared in four inbred strains of mice (DBA/2J; C3H/HeJ; AKR/J; C57BL/6J). Basal rates of ethanol elimination were determined in individual mice after intraperitoneal injections of ethanol. Little variability in this basal rate of ethanol elimination was observed within each strain. Mice were then exposed to ethanol vapor for 4 hours. In both injected and treated mice the dose of ethanol was varied to produce blood ethanol levels ranging from 50 to 250 mg%. Ethanol elimination increased maximally 1.5 to 4-fold in all four strains following 4 hours of vapor treatment at the same blood ethanol level; however, the dose at which the maximal increase occurred differed among the strains. DBA/2J mice exhibited a maximal increase in the rate of ethanol elimination when ethanol concentrations were in the range of 30 to 50 mg%; the increase was smaller as the dose was increased. In contrast, AKR/J and C57BL/6J mice required 100 to 150 mg% ethanol to activate SIAM. These data indicate clearly that the SIAM effect is a common phenomenon, and that dose-response relations differ in various inbred strains of mice.

Strain differences Alcohol metabolism Mice Ethanol

EVIDENCE exists that ethanol metabolism in humans is under genetic control. For example, monozygotic twins have very high concordance rates for ethanol elimination [9]. The biological system(s) responsible for this observation, however, remain to be identified. Presumably one or more rate-determining factor in ethanol metabolism is under genetic regulation.

Chronic treatment with ethanol increased the rate of ethanol elimination in rats [2,7] and alcoholics [6]. This increase in the rate of ethanol elimination has also been demonstrated within several hours after one acute dose of ethanol in rats *in vivo* and in the isolated, perfused rat liver [8,11]. This phenomenon has been defined as the swift increase in alcohol metabolism (SIAM). SIAM is thought to result from a stimulation of ATPase activities by ethanol which leads to enhanced electron flux in the mitochondrial respiratory chain. This increases the rate of respiration and turnover of NADH which in turn accelerates the rate of ethanol metabolism (Fig. 1).

The SIAM effect has been shown to be heritable in the rat [8]; however, genetic analysis in the rat is expensive and time consuming. Therefore, the present study was initiated to determine if SIAM mutants exist in one or more inbred strains of mice.

METHOD

Mouse Strains

Male mice from four inbred strains (DBA/2J; C3H/HeJ; AKR/J; C57BL/6J) employed in these studies were obtained from the Jackson Laboratory, Bar Harbor, ME. They were all well-fed adults between 25–35 g and were eight to twelve weeks old.

Determination of Breath Ethanol Concentrations

Blood ethanol concentrations were calculated from measurements of breath ethanol. Individual mice were forced to breathe in a 2.75 ml closed vessel for 17 sec to ensure maximal equilibration between breath and vapor in the breathalyzation chamber. After equilibration, a 1.0 ml sample of exhaled breath was injected into a gas chromatograph. The gas chromatograph used was a Hewlett-Packard Model 5720 equipped with a flame ionization detector. The apparatus contained a six-foot long by 1/4 inch carbowax 60/80 column. The operating parameters for all ethanol determinations were as follows: oven, 110°C; detector, 250°C; injection port, 250°C; carrier gas flow rate, 80 ml/min. A peak corresponding to ethanol with a retention time of about 50 seconds was compared with ethanol standards [12]. Ethanol concen-

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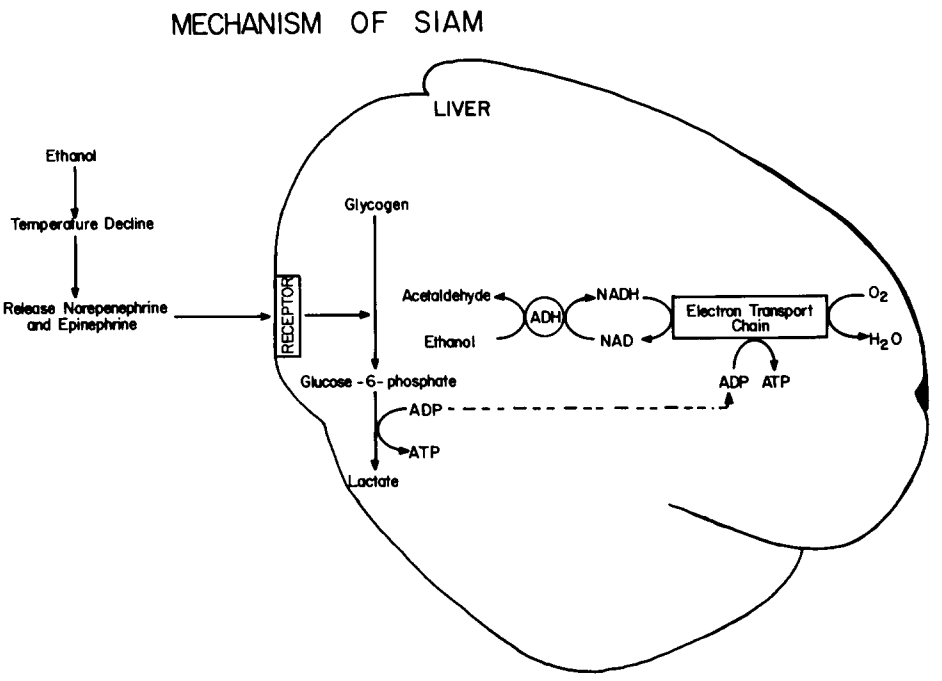

FIG. 1. Scheme depicting the swift increase in alcohol metabolism. Ethanol causes a release of catecholamines and stimulates the release of glycogen. As ethanol is continually introduced into the liver, glycogen reserves are depleted and a build-up of ADP occurs. The ADP enters the mitochondrion and stimulates oxygen uptake and the reoxidation of NADH.

trations in blood correlated linearly with breath ethanol (data not shown). The concentration of ethanol vapor in the inhalation chamber was also measured routinely by gas chromatography.

Treatment with Ethanol

Adult male mice received various doses of ethanol intraperitoneally. Basal rates of ethanol elimination were determined by taking multiple breath samples every 20 to 30 min (see Fig. 2). Other groups of mice were placed in Plexiglas chambers (50×50×40 cm) with free access to food and water and exposed to various concentrations of ethanol vapor for periods ranging from 0 to 8 hours (see Figure legends for details). At the end of the vapor treatments, rates of ethanol elimination were determined without injecting additional ethanol (Fig. 2).

For some experiments, groups of mice were given ethanol either by injecting different doses (<0.5–3.0 g/kg) intraperitoneally or by varying the flow rate of ethanol vapor into the chamber to obtain blood ethanol ranges from <50 to 250 mg%.

Statistical comparisons were made using Student's *t*-test for non-paired data.

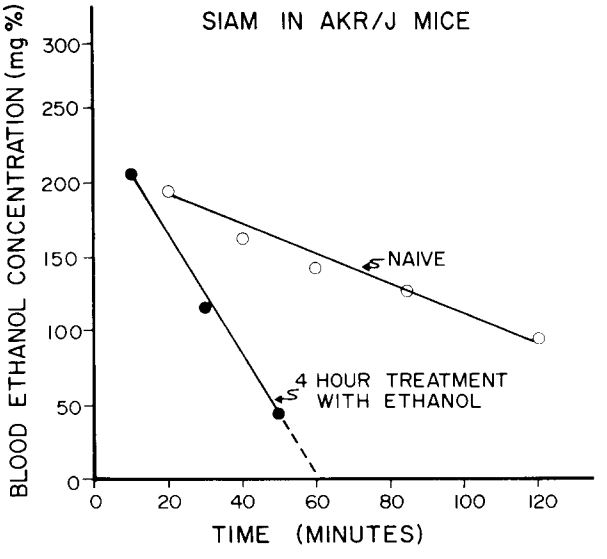

FIG. 2. Example of the swift increase in alcohol metabolism in the AKR/J mouse. Ethanol (2.0 g/kg, IP) was given and a rate of ethanol elimination was determined by taking repeated breath samples during 2 hours (see Method section). Mice were also placed in a Plexiglas chamber filled with ethanol vapor. After treatment for 4 hours, the mice were removed from the chamber and the rate of ethanol elimination was again determined.

RESULTS

The Swift Increase in Alcohol Metabolism (SIAM)

A typical experiment of the type carried out in this study is presented in Fig. 2 for mice from the AKR/J strain. Two

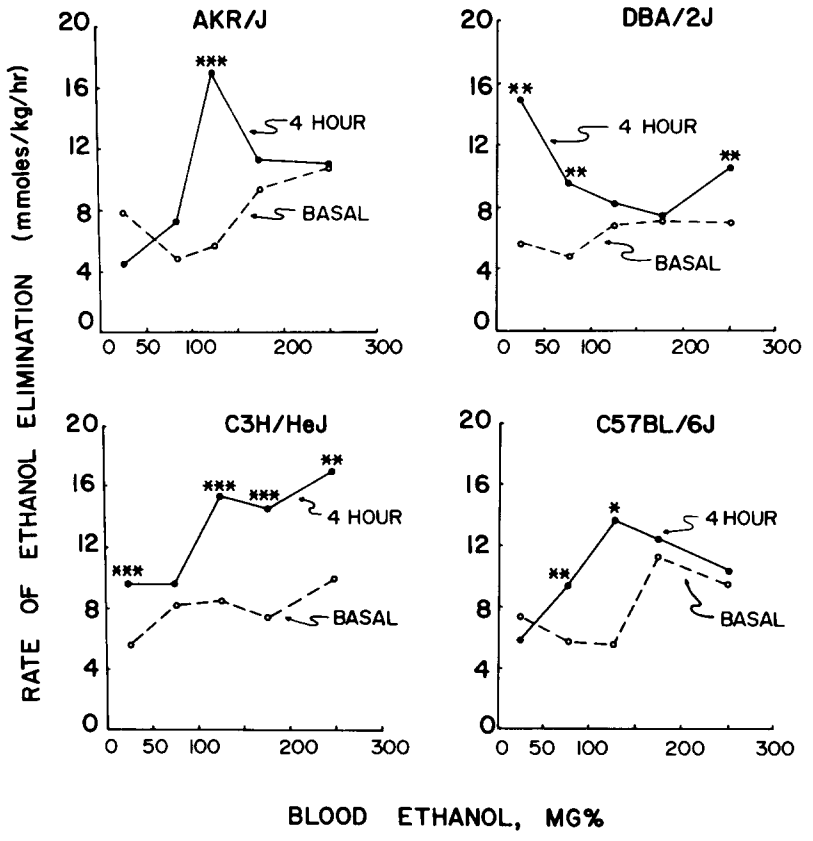

FIG. 3. Ethanol elimination in four inbred mouse strains. Mice were given different doses of ethanol (<1 to 3.5 g/kg, IP) and the basal rate (●- - -●) of ethanol elimination was determined as in Fig. 2. Rates are plotted at the mid-point of each dose range. Mice were then exposed to different concentrations of ethanol vapor for four hours. Rates of elimination (●—●) were determined as described in Methods. *$p<0.05$; **$p<0.01$; ***$p<0.001$ are for the comparison between the lowest rates and rates from the other strains at any given dose range of ethanol.

groups of mice were treated with ethanol; one group received 2.0 g/kg ethanol by intraperitoneal injection while the other group received ethanol vapor for 4 hours. Breath samples were collected at 15–20 min intervals for 3 hours from both groups and the rate of ethanol elimination was calculated from the linear decline in ethanol concentration per unit time. Mice treated with ethanol vapor for four hours eliminated ethanol at rates 2.5 times faster than those receiving ethanol acutely by injection (5.6±0.9 vs. 13.6±0.5 mmoles ethanol/kg/hr). This increase in rate of elimination following exposure to ethanol is defined as SIAM. Previous data have shown that the SIAM effect can also be elicited by repeated injections in the rat [11].

Effect of Dose of Ethanol on the Basal Rate of Ethanol Elimination

Groups of mice were given injections of various doses (<1 to 3.5 g/kg) of ethanol intraperitoneally to achieve blood ethanol ranges of <50, 50–100, 100–150, 150–200, or 200–300 mg%. With blood ethanol levels less than 150 mg%, all strains of mice eliminated ethanol at relatively constant basal rates (6 to 8 mmoles/kg/hr) independent of the ethanol concentration (Fig. 3, dashed line); however, with blood ethanol concentrations above 150 mg%, C57BL/6J, AKR/J, and C3H/HeJ mice had significantly higher rates. This increase was not, however, observed in mice of the DBA/2J strain (Fig. 3, dashed line).

Similar ranges of blood ethanol were achieved by treating mice in the vapor chamber for 4 hours (Fig. 3, solid line). Mice of the DBA/2J strain exhibited maximal rates of ethanol elimination after four hours of exposure to <50 mg% ethanol; higher doses produced lower rates of ethanol elimination. In contrast, mice of the other three strains required approximately 150 mg% ethanol to produce the maximal SIAM effect. At the highest dose (250 mg%), rates of ethanol elimination decreased in AKR/J and C57BL/6J but were increased in C3H/HeJ mice (Fig. 4).

Figure 4 shows the SIAM effect in various blood ethanol concentration ranges in the four mice strains. The data indicate clearly that the dose of ethanol required to trigger SIAM varies in the different strains. For example, in mice of the DBA/2J strain, a 2-fold increase was observed at very low blood levels (<50 mg%) of ethanol (Fig. 4); the increase in

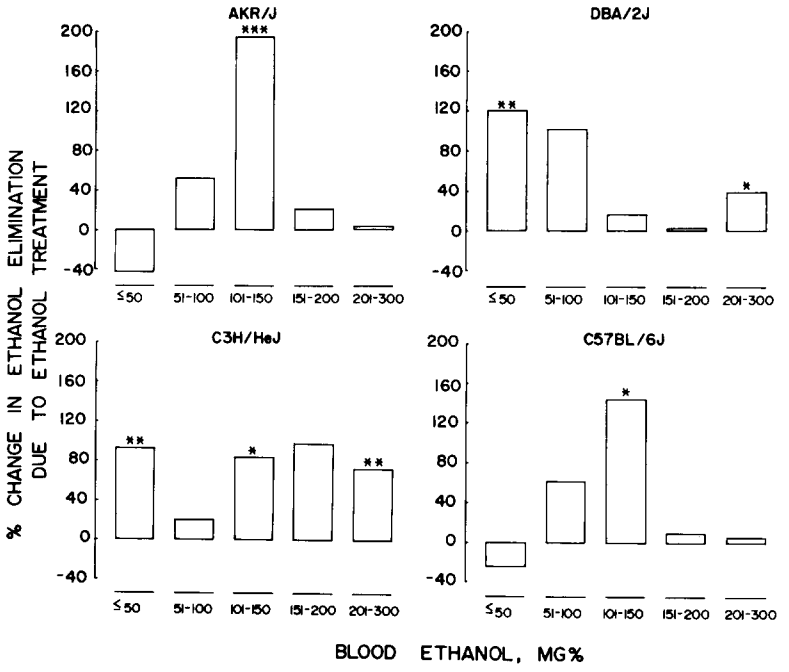

FIG. 4. SIAM effect as a function of the level of blood ethanol in four inbred mouse strains. Percentage difference between basal and treated rates from Fig. 3.

rate was progressively smaller as the dose of ethanol was increased. In contrast, 100–150 mg% ethanol was required to activate the SIAM effect maximally in AKR/J, C57BL/6J and C3H/HeJ mice. Very high blood levels (200–300 mg%) of ethanol caused a decline in the SIAM effect in the AKR/J and C57BL/6J strains whereas high rates were maintained at elevated ethanol levels in C3H/HeJ mice (Fig. 4).

DISCUSSION

Basal rates of ethanol elimination in the four inbred strains of mice compared in this study were not statistically significantly different at low doses of ethanol (e.g., doses up to 150 mg%). However, at higher doses there was a tendency for some of the strains to have higher rates of ethanol elimination (Fig. 3). This was true for C57BL/6J, AKR/J and C3H/HeJ mice. On the other hand, mice of the DBA/2J strain did not alter their basal rate of ethanol elimination significantly at higher concentrations of ethanol studied. The fact that basal rates of ethanol elimination are elevated at higher doses of ethanol was observed previously in the rat [12] and could be due to an immediate activation of the SIAM phenomenon at high doses or to an increase in minor pathways of ethanol elimination which require high concentrations of ethanol to operate.

These studies conclusively demonstrate that the SIAM effects exists in four inbred strains of mice. Patterns of the SIAM response differed markedly when the DBA/2J and C3H/HeJ strains were compared with the AKR/J and C57BL/6J strains. For example, the SIAM effect was observed at very low ethanol concentrations (<50 mg%) in DBA/2J mice. In contrast, blood levels of at least 150 mg% ethanol were required to activate the SIAM effect maximally in the mice of the AKR/J and C57BL/6J strains; however, maximal increases of about two-fold were observed in all strains studied.

The fact that the SIAM effect is dose-dependent may explain why some workers have not observed this phenomena. For example, Braggins and Crow [1] did not observe SIAM using randomly outbred rats. They concluded that the SIAM effect was an artifact caused by intragastric administration. This conclusion can be ruled out, however, since the SIAM effect was elicited in this study by vapor (Fig. 4) and in other studies by injection (data not shown). Because complicated time and dose relationships exist with respect to the SIAM phenomenon, it is possible that Braggins and Crow did not observe the SIAM effect because they chose inappropriate experimental conditions. In addition, our work indicates that the specific genetic makeup of the test animals appears to be an important variable, a fact not considered by Braggins and Crow [1].

There is the question of why the SIAM effect eventually declines with higher doses (Fig. 4). In mice of the C57BL/6J, C3H/HeJ and AKR/J strains, the basal rate of ethanol elimination increased at higher doses of ethanol due possibly to a rapid SIAM effect. Thus, an apparent decline in the SIAM effect could be due to the higher basal rates in these strains thereby reducing the difference between basal and treated rates. In one case, however, this is clearly not true. DBA/2J mice showed a decrease in the SIAM effect at higher levels of blood ethanol, yet the basal rates were relatively constant at all ranges of blood ethanol studied. It is possible that tachyphalyxis to hormones responsible for the SIAM effect occurs (Fig. 1).

The SIAM effect is most likely due to an increased rate of reoxidation of NADH which increases alcohol

dehydrogenase-dependent ethanol metabolism (Fig. 1) based on previous work by Yuki and Thurman [11] and Yuki *et al.* [10]. These data indicate that at least part of the stimulated oxygen uptake after treatment with ethanol results from lower rates of glycolytic ATP generation. The ADP that is not phosphorylated in the cytosol then enters the mitochondrion where it stimulates oxygen uptake. Thus, it is the stimulation of electron transport and oxygen uptake that leads to a faster rate of reoxidation of NADH to NAD^+ resulting in an acceleration of alcohol dehydrogenase-dependent ethanol metabolism; this results in the SIAM effect.

We may sepculate on the potential significance of this data to alcohol-related liver disease. First, it is well known that alcoholic liver cirrhosis occurs in only a small percentage of chronic alcohol abusers suggesting that predisposing factors leading to cirrhosis may be heritable [5]. The present study indicates that the dose of alcohol required to produce the SIAM effect varies with the genotype (Fig. 4). It follows from this observation that a given dose of ethanol could activate ethanol metabolism via the SIAM mechanism in one individual but not in another. This is potentially important with respect to liver cirrhosis since ethanol elimination requires the reoxidation of NADH and the uptake of oxygen via the mitochondrial electron transport chain [7]. Higher rates of ethanol elimination due to the SIAM effect would require higher rates of oxygen uptake and therefore produce a steeper oxygen gradient between different regions of the liver lobule. Israel and his colleagues [3] showed that chronic treatment with alcohol and then brief exposure to hypoxia exacerbated liver damage and suggested that the increase in hepatic oxygen uptake due to ethanol [2,11] causes the oxygen gradient in the liver lobule to become steeper. The hypothesis states that this leads to hypoxic damage in the centrilobular region and ultimately to hepatic inflammation, fibrosis and cirrhosis. We have recently obtained direct evidence with miniature oxygen electrodes placed on periportal and pericentral regions of the liver that ethanol indeed steepens the hepatic oxygen gradient [4]. Thus, it is tempting to speculate that an individual possessing a SIAM effect may be more likely to develop cirrhosis of the liver as a result of hypoxic damage due to ethanol than someone lacking the effect because of his unique genetic make-up. More work will be needed in the future to evaluate this interesting idea.

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