Involvement of Hormones in the Swift Increase in Alcohol Metabolism*†

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ABSTRACT

The purpose of this study was to determine the time course of changes in blood levels of various hormones in C57BL/6J mice during exposure to ethanol vapor. Groups of adult male mice were given 2.0 g per kg ethanol intraperitoneally or as continuous vapor for four hours and rates of ethanol elimination were measured. In parallel, blood samples were collected at timed intervals over 5.5 hours during and following exposure to ethanol. Blood levels of epinephrine, norepinephrine, corticosterone, and glucagon were elevated two- to four-fold during ethanol treatment and declined to basal values within one hour following termination of treatment. Elevated blood levels of epinephrine, norepinephrine and corticosterone were highly correlated with higher rates of ethanol elimination (r = 0.80, 0.78, and 0.72, respectively). In contrast, thyroxine and insulin levels were not affected by ethanol. These findings are consistent with the idea that acute administration of ethanol causes the release of glycogenolytic hormones which in turn increase rates of ethanol metabolism.

Introduction

Evidence relevant to the question of whether or not hormones are involved in the regulation of ethanol metabolism comes from studies with inbred strains of rat. These studies have shown that castration or treatment with estradiol in males raises both the rate of ethanol metabolism and the specific activity of alcohol dehydrogenase.2,14 Testosterone appears to have an opposite effect in females.17 In addition, it has been observed that thyroidectomy leads to an increase in hepatic alcohol dehydrogenase (ADH) activity without any corresponding increase in the rate of ethanol metabolism,15 while rats acclimatized to cold conditions are reported to metabo-
lize ethanol more quickly, despite a small reduction in liver (ADH) activity. In a previous study, Yuki and Thurman, using the perfused rat liver model, showed that ethanol uptake was nearly doubled 2.5 hours after treatment with ethanol. They also demonstrated that epinephrine given one hour prior to liver perfusion increased hepatic oxygen consumption by about 40 percent. In addition, the effects of ethanol and epinephrine were not additive, suggesting that they may have common mechanisms of action. The increase in the rate of ethanol metabolism was inhibited largely by 4-methylpyrazole, an inhibitor of ADH, indicating that ADH is involved in the Swift Increase in Alcohol Metabolism (SIAM).

Conclusive evidence in support of this hypothesis has come from studies comparing ADH-positive and negative deer-mice. At any given dose of ethanol, vapor treatment with ethanol increased rates of ethanol metabolism in ADH-positive, but not ADH-negative deer-mice. Additional studies using alpha and beta adrenergic blocking agents demonstrated a decrease in hepatic oxygen consumption and a significant reduction in the rate of ethanol elimination in perfused rat liver indicating that adrenergic hormones play an important role in regulation of alcohol elimination. The SIAM phenomenon has been demonstrated in vivo in rats, in several inbred strains of mice, the ADH-positive deer-mouse, and humans.

Based on previous observations, it is reasonable to propose that an early key element in the sequence of events leading to SIAM may be the release of hormones by ethanol. In support of this hypothesis, SIAM can be obtained by the administration of adrenergic hormones and blocked by alpha and beta antagonists, adrenalectomy and hypophysectomy.

While several studies have been performed to examine the effects of hormones administered over several days on enzymes involved in ethanol metabolism and rates of ethanol elimination, little is known concerning short-term effects of ethanol treatment on blood hormone levels. The purpose of this study was to investigate the effect of ethanol administration on the time course of changes in circulating levels of adrenergic and glycogenolytic hormones, and to determine possible relationships between circulating hormone levels and rates of ethanol elimination. Preliminary accounts of this work have appeared elsewhere.

Materials and Methods

ANIMALS

Male inbred mice (C57BL/6J) were obtained from the Jackson Laboratory.* Animals were well fed adults between 25 to 38 g and eight to 12 weeks of age. Mice were maintained on a lab chow diet and given water ad libitum.

DETERMINATION OF BREATH ETHANOL CONCENTRATIONS

Blood ethanol concentration were calculated from measurements of breath ethanol as described in detail elsewhere. Individual mice were forced to breathe for 15 to 20 seconds into a 2.75 ml closed vessel maintained at 37°C in order to ensure complete equilibration between breath and chamber vapor. A 1.0 ml sample of chamber vapor was injected into a gas chromatograph (Model 5720† equipped with a flame ionization detector. Ethanol was separated on a carbowax 60/80 column (6 feet

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long by ¼ inch). Operating parameters for all ethanol measurements were: oven—110°C; detector—315°C; injection port—300°C; and gas flow—80 ml per min. Ethanol concentrations in blood correlated well with breath ethanol (data not shown). Rates of ethanol elimination were calculated from the linear decline in blood ethanol concentrations by the method of Widmark.23

TREATMENT WITH ETHANOL

Mice received two g per kg ethanol in saline by intraperitoneal injection, or were exposed for four hours to 15 to 30 mg per l ethanol vapor in a Plexiglass inhalation chamber (50 × 50 × 40 cm). After treatment, rates of ethanol elimination were determined. Four hours of vapor treatment was chosen to achieve maximal increases in ethanol metabolism over basal values.24

ANALYSIS OF PLASMA HORMONES

Animals were decapitated rapidly within 30 seconds after removal from their respective chambers. Blood was collected in heparinized tubes and centrifuged immediately at 2000 × g for 15 minutes at 4°C. Epinephrine and norepinephrine in plasma were assayed in triplicate employing a radioenzymatic procedure,4 and plasma levels of thyroxine, corticosterone, glucagon and insulin were quantitated in duplicate using specific radioimmunoassay techniques.5

STATISTICAL ANALYSIS

Correlations between rates of ethanol elimination and plasma hormone levels were analyzed by a modified linear regression method.3 Data from the animal studies were further analyzed by Student's paired-t test.

RESULTS

A typical experiment describing the protocol used in this study is illustrated in figure 1. Mice were placed in a Plexiglass chamber and exposed to ethanol for four hours as described in "Materials and Methods". At various times, animals were removed and breath samples were taken. Blood ethanol concentrations typically ranged from 5 to 15 mmol per l for the first 2.5 hours of vapor treatment and increased to about 27 mmol per l after four hours of exposure (figure 1). Breath samples were taken over the next 1.5 hours and rates of ethanol elimination were calculated. Rates of ethanol elimination ranged from 5 to 18 mmol per kg per h and were consistent with previous data obtained with the C57BL/6J mouse.20

Circulating levels of plasma epineph-
HORMONES AND ETHANOL METABOLISM

Circulating epinephrine increased from six to 18 ng per ml during the first two hours of ethanol treatment (figure 2). This represented an increase of about 300 percent compared to the basal state. Control mice placed in Plexiglass chambers and handled in a similar manner, but not exposed to ethanol, showed only a slight increase in circulating epinephrine of approximately 30 percent, presumably related to the stress of handling.

After two hours of ethanol treatment in a vapor chamber, plasma epinephrine values declined toward basal levels and continued to decline for 1.5 hours after the vapor treatment was discontinued (figure 2). Similar results were obtained for norepinephrine (not shown). Plasma norepinephrine was elevated to eight ng per ml from a basal level of three ng per ml within one hour after exposure to ethanol and remained elevated until exposure to ethanol vapor was discontinued. Plasma corticosterone levels were increased three-fold during ethanol administration, reaching peak values in less than 60 minutes and declining to basal values after ethanol exposure was interrupted (figure 3). In contrast, concentrations of thyroxine and insulin were unaffected by ethanol treatment. In table I are illustrated the average basal hormone values and maximum increases observed during acute ethanol treatment. Plasma epinephrine, norepinephrine, corticosterone, and glucagon concentrations were increased significantly from control levels (up to 400 percent after exposure to ethanol vapor.

The statistically significant correlations between maximal changes in plasma epinephrine, norepinephrine, corticosterone and glucagon levels and rates of ethanol elimination (r = 0.80, P < 0.005, 0.78, P < 0.001, 0.72, P < 0.001, 0.52, P < 0.05, respectively) support the hypothesis that adrenergic and glycogenolytic hormones may play a role in SIAM (table I, figure 4).

Discussion

Data presented here indicate that after four hours of vapor treatment with

![Figure 2. Plasma Epinephrine Concentrations During and Following Treatment with Ethanol Vapor. C57BL/6J mice were exposed to ethanol vapor (15 to 30 mg per l) as indicated by the solid line. Control mice exposed to air are denoted graphically by a dashed line. Each point represents the mean of duplicate analysis.](figure2)

![Figure 3. Plasma Corticosterone Concentrations During and Following Treatment of Mice with Ethanol Vapor. Conditions as in figure 2. Corticosterone was measured by a standard radioimmunoassay procedure. Data represents averages of two mice per time point.](figure3)
ethanol or intraperitoneal injection of ethanol plasma concentrations of epinephrine, norepinephrine, corticosterone and glucagon were elevated two- to four-fold above basal values. This treatment also increased rates of ethanol elimination significantly in C57BL/6J mice.

It is well known that alcohol causes a release of epinephrine and norepinephrine from the adrenal medulla. It is also well established that epinephrine and glucagon cause glycogenolysis and elevate blood glucose. As reported by Yuki and Thurman, the acute administration of ethanol to mice leads to decreased rates of glycolysis, increased glucose output, and increased rates of oxygen uptake. Moreover, alcohol treatment elevated levels of circulating catecholamines at times (2.5 h) when the enhanced effects on oxygen uptake were observed. Therefore, adrenergic and glycogenolytic hormones may mediate the sequence of events responsible for the increased rate of ethanol metabolism. A possible explanation is that hormone-stimulated glycogenolysis leads to depletion of tissue carbohydrate reserves (glucogen), which in turn causes glycolysis to decline. Glycolysis is an adenosine-tri-phosphate (ATP)-producing reaction, so liver adenosine diphosphate (ADP) not phosphorylated via glycolysis enters the mitochondria and is phosphorylated by the electron transport chain with a concomitant increase in oxygen consumption. As a consequence, reduced nicotin-

![Figure 4. Correlation Between Maximal Concentrations of Epinephrine in Plasma and Rates of Ethanol Elimination. Maximal values of plasma epinephrine levels were plotted against rates of ethanol elimination and a correlation was derived using a least-squares linear regression analysis.](image)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Basal Plasma*</th>
<th>Maximal Plasma</th>
<th>Percent Increase</th>
<th>Correlation with Ethanol Elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>4.4 ng/ml</td>
<td>17.2 ng/ml</td>
<td>391.0</td>
<td>0.80 &lt;0.005</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>3.1 ng/ml</td>
<td>7.8 ng/ml</td>
<td>252.0</td>
<td>0.78 &lt;0.001</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>303.0 ng/ml</td>
<td>820.0 ng/ml</td>
<td>271.0</td>
<td>0.72 &lt;0.001</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>5.7 ng/ml</td>
<td>6.0 ng/ml</td>
<td>5.0</td>
<td>-0.21 NS</td>
</tr>
<tr>
<td>Glucagon</td>
<td>314.0 pg/ml</td>
<td>529.0 pg/ml</td>
<td>168.0</td>
<td>0.52 &lt;0.05</td>
</tr>
<tr>
<td>Insulin</td>
<td>19.3 μU/ml</td>
<td>15.2 μU/ml</td>
<td>&lt;5.0</td>
<td>-0.17 NS</td>
</tr>
</tbody>
</table>

*Before ethanol treatment
NS = Not Significant
C57BL/6J mice were exposed to ethanol vapor (15 to 30 mg/l) for four hours, and rates of ethanol elimination were determined following withdrawal of vapor treatment as described in the Materials and Methods section. Maximal concentrations of plasma hormones were correlated with rates of ethanol elimination using a least-squares linear regression analysis. Six points per hormone were used for the regression calculation.
amide-adenine dinucleotide (NADH) is reoxidized at a faster rate, thereby providing more nicotinamide-adenine dinucleotide\(^+\) (NAD\(^+\)) for the alcohol dehydrogenase (ADH) reaction and an enhanced rate of ethanol elimination.\(^{26}\)

As illustrated in figure 5, this normally requires the mediation of hydrogen shuttles capable of moving reducing equivalents from cytosol to mitochondria and can also be influenced by hormones, enzymes, and other factors involved in the regulation of ethanol oxidation.

Acute ethanol administration appears to stimulate glucagon release (Table I). It has been proposed that the glycojenolytic effect of glucagon is similar to that produced by the catecholamines with a concomitant increase of liver phosphorylase activity and rapid decrease in liver glycogen content.\(^{11}\) Thus, elevated plasma levels of glucagon may contribute to the depletion of hepatic glycogen and activation of the SIAM response. In contrast, other hormones which affect glycogen utilization directly such as insulin, or indirectly as thyroxine, were unaffected by ethanol treatment. Thus, these latter two hormones do not appear to be involved in SIAM.

Plasma corticosterone levels were elevated rapidly and significantly by ethanol treatment (figure 3) and demonstrate an immediate response to alcohol-induced stress.\(^{12}\) It is not clear what role corticosterone might play in SIAM.

Adrenergic and glycogenolytic hormones may also activate peripheral lipolysis of adipose stores leading to elevation of circulating free fatty acids and enhancement of the "peroxidatic" reaction of catalase for ethanol.\(^{18}\) It has been demonstrated recently that peroxisomal \(\beta\)-oxidation of fatty acids provides \(\text{H}_2\text{O}_2\) for the peroxidation of ethanol by catalase-\(\text{H}_2\text{O}_2\).\(^9\) Handler et al\(^{10}\) demonstrated elegantly that rates of \(\text{H}_2\text{O}_2\) generation in the perfused livers from ADH-negative deermice were sufficient to support ethanol metabolism via catalase at rates up to 60 \(\mu\text{mol}\) per g per h, about three-fourths of the rate in perfused livers from ADH-positive deermice. They concluded that catalase-
dependent ethanol metabolism could occur significantly in perfused livers if adequate substrate for \( \text{H}_2\text{O}_2 \) generation (i.e., albumin-bound fatty acids for peroxisomal \( \beta \)-oxidation) was provided. Thus, it is conceivable that a portion of the SIAM response is attributable to increased peroxidation of ethanol via catalase caused by higher rates of \( \text{H}_2\text{O}_2 \) generation from elevated levels of fatty acids released from adipocytes by lipogenic hormones (figure 5).

Since specific adrenergic and glycogenolytic hormones are elevated by acute ethanol treatment with a time course similar to SIAM, short-term increases in rates of ethanol metabolism may be a consequence of hormone-induced changes in liver intermediary metabolism. The marked changes in plasma hormones over time may also explain why the dose and time relationships in SIAM are so complex. For example, if plasma epinephrine levels increase slowly after exposure to ethanol, a longer period of time will be needed to achieve the maximum SIAM response. In contrast, plasma epinephrine and corticosterone levels increased significantly within one to two hours following exposure to ethanol and then declined rapidly, resulting in a sharp increase followed by a swift decrease in ethanol elimination to basal values. The differential alterations in circulating hormone levels may explain the varied SIAM responses seen in rats and different inbred strains of mice.

Acknowledgment

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References


