Mechanism of the alcohol cyclic pattern: role of catecholamines

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The urinary alcohol cycle seen in the intragastric ethanol feeding rat model of early alcoholic liver disease has been shown to be thyroid hormone dependent (8). The urinary alcohol level (UAL) cycle was prevented by propylthiouracil treatment and pituitary stalk severance (8) or by treatment with a high dose of thyroxine (7). However, a high dose of thyroxine given with ethanol led to centrilobular ischemic necrosis (7). The necrosis was thought to be due to hypermetabolic state induced by the combination of hyperthyroidism and high blood alcohol levels (BAL). Liver hypoxia was suspected at high BAL at the peak of the UAL cycle because ATP levels were reduced and the NADH/NAD ratio shifted to the reduced state at the peaks (1). The idea that hypoxia occurred at the UAL peaks was supported by the increase in hypoxic indicators such as erythropoietin and VEGF upregulation and an increase in hypoxyprobe labeling of hepatocytes measured by immunofluorescence (1). Thyroxine treatment prevented the UAL cycle by increasing the metabolic rate as was indicated by the increased rate of ethanol elimination observed (7).

To further investigate the mechanism involved in the UAL cycle and centrilobular necrosis seen in the intragastric feeding model, rats were fed ethanol together with the adrenergic drug ephedrine and caffeine as used in weight loss dietary regimens (2–4). Similar dietary regimens have occasionally caused hepatic toxicity, but the nature of the toxicity was not defined (5). Adrenergic drugs are known to increase the metabolic rate and thus accelerate the ethanol elimination rate (9) as was observed during the UAL cycle (10). Caffeine enhances the adrenergic effect on metabolism, increasing cAMP levels induced by adrenergic signaling. It does this by inhibiting the breakdown of cAMP by phosphodiesterase. Thyroid hormone treatment also accelerates the ethanol elimination rate (7). Thyroid hormone potentiates the ability of catecholamines to increase the rate of O2 consumption (3). In the UAL cycle, a cyclic increase in the rate of O2 consumption was observed despite a constant rate of ethanol feeding (8). Thus the data are consistent with the hypothesis that the combination of ethanol and catecholamine feeding will, like thyroxine, accelerate the rate of ethanol elimination and thus block the UAL cycle. The results of this report further support the hypothesis, deeming it to be correct.

MATERIALS AND METHODS

Male Wistar rats weighing 300 g (Harlan Sprague Dawley, Hollister, CA) were fed diet and ethanol intragastrically continuously 24 h/day for 6 wk together with pair-fed controls fed dextrose isocaloric to ethanol (groups 3 and 4, respectively). Two other groups were fed the diet supplemented with ephedrine (50 mg·kg−1·day−1) and caffeine (175 mg·kg−1·day−1). One of these groups was fed ethanol (group 1), and one was pair-fed isocaloric dextrose (group 2). All four groups had five rats per group. Ethanol was fed at a...
constant rate of 13 g·kg⁻¹·day⁻¹ at which 40% of the total calories were derived from ethanol or isocaloric dextrose. The diet fed was previously described (8). Of total calories 28.9% were derived from fat, 6.2% from dextrose, and 24.9% from protein.

UALs were measured daily over the terminal 20- to 25-day test period. Urine was collected over a 24-h period. Urine was protected from evaporation by an overlay of toluene. UAL was measured colorimetrically (QED Saliva Alcohol Test A150; ST Technologists, Bethlehem, PA). Blood levels of serum alanine aminotransferase were measured by using a clinical analyzer (a kinetic rate method on Synch Roncx Systems; Beckman Instruments, Brea, CA). Total blood catecholamines were measured by HPLC (Quest Diagnostics, San Juan Capistrano, CA). Body weights were recorded weekly for 6 wk of feeding. Liver weight-to-body weight ratios were calculated at the time of death.

During the terminal 20- to 25-days, the UAL were charted to determine whether the UAL cycle was manifest and to determine the ethanol dose each rat could eliminate beginning at day 15 of recording the UAL cycle. On day 15, the dose of ethanol was increased serially every few days to 14, 15, 16, and 17 g·kg⁻¹·day⁻¹ to the dose, which proved lethal to the rat because of overdose. In this way, the dose of ethanol...
that could be eliminated by each rat was determined. At the time of death or in the case of controls death, the liver tissue was fixed in zinc formalin (10%) and processed for histological examination by using hematoxylin and eosin and reticulin staining.

The research protocol was approved by the Research and Education Institute Animal Care Committee in accordance with the guidelines for animal care as described by the National Academy of Sciences (1996).

Statistical methods employed were ANOVA and all pairwise multiple group comparisons (Student-Newman-Keuls method).

RESULTS

Weight gain or loss in rats from the four experimental groups is shown in Fig. 1. Note that the rats fed ethanol or pair-fed dextrose gained weight, whereas the rats fed ephedrine and caffeine in their diet lost weight. This was despite the fact that they received the same amount of calories over 24 h as the pair-fed rats. The weight loss was even greater when the rats were fed ephedrine + caffeine + ethanol compared with rats pair-fed ephedrine + caffeine + dextrose ($P < 0.001$) (Figs. 1–2). The change in body weight over the 6-wk feeding period was significantly different (Fig. 2) ($P < 0.001$). The starting weight for all four groups was not different (Figs. 1–2).

End body weights for the treated groups 1 and 2 were statistically different when compared with the untreated groups ($P < 0.001$) (Fig. 2). These differences establish the weight loss effect of the ephedrine and caffeine diet treatment when the rats were pair-fed, indicating that catecholamine treatment increased the metabolic rate in the ephedrine and caffeine treated rats. Ethanol + ephedrine + caffeine further increased the weight loss presumably because the blood catecholamines were high in this group.

The liver weight-to-body weight ratio was calculated as a measure of relative liver enlargement (Fig. 3). The ratio was significantly increased when ethanol was fed with ephedrine and caffeine (group 1 compared with groups 2–4) ($P < 0.001$). The ratio was also increased in the ethanol-fed rats compared with the rats pair-fed dextrose ($P < 0.001$) (Fig. 3). The liver weight not corrected for body weight shows essentially the same pattern (Fig. 4).

Blood catecholamines were determined in all four groups (Fig. 5, A and B). The blood catecholamine levels were elevated by ethanol + ephedrine + caffeine (group A), by ethanol alone (group C), and by ephed-
rine + caffeine alone (group B). The blood catecholamine levels were total catecholamines, which included epinephrine and norepinephrine but not dopamine. The elevation of the catecholamines in the ephedrine + caffeine-fed group (B) was less than the ethanol-fed group (group C). The combination of ethanol and ephedrine + caffeine-fed group (group A) had significantly higher catecholamines than the ephedrine + caffeine or ethanol-fed group (groups B and C). When the levels of groups B and C were added (group E), and compared with the combination of ethanol and ephedrine + caffeine (group A), group A values were
significantly higher. This indicates a synergistic effect when both ethanol and ephedrine + caffeine were fed together. Epinephrine constituted more than half of the total catecholamines except for group A where norepinephrine predominated (Fig. 5B).

Liver damage was assessed by measuring serum alanine amino transferase (ALT) and examining the histopathology of the liver in all four groups at the beginning and end of the experiment. There was a significant increase in the ALT levels in rats fed ethanol with ephedrine and caffeine (group 1) when compared with rats fed ethanol alone without treatment (group 3) (P < 0.001). Rats fed ethanol without treatment and the pair-fed controls (group 3 vs. group 4) were also significantly different (P < 0.001) (Fig. 6).

The histopathology of livers in the four experimental groups differed (Fig. 7). Ethanol fed without treatment (group 3) showed steatohepatitis (Fig. 7) compared with the normal histology of the pair-fed control (group 4) (Fig. 7A). One liver in group 3 had one small focus of centrilobular necrosis. The liver cords were narrowed by ephedrine and caffeine with and without ethanol feeding (groups 1 and 2) (Fig. 7, C and D). In contrast to the livers of rats fed ethanol without treatment (group 3) the livers of rats fed ethanol with ephedrine and caffeine (group 1) showed only minimal microvesicular fat. Instead, four of the rats showed focal centrilobular ischemic necrosis (Fig. 7, E–G). One rat in group 1 also showed blood-filled cysts without endothelial lining characteristic of peliosis hepatitis (Fig. 7H). One rat in group 1 also showed scars, which presumably were a result of prior episodes of ischemic necrosis (Fig. 8, A–F).

Thus the ephedrine and caffeine diet prevented ethanol-induced steatohepatitis, but the livers in this group (group 1) showed centrilobular ischemic necrosis. Otherwise, the five livers in this group resembled the pair-fed ephedrine and caffeine control livers (group 2).

The UAL cycle was present when the UALs were measured over the last 15 days of the 6-wk feeding period studied in group 3 (Fig. 9). When the ethanol dose was increased from 13 g·kg⁻¹·day⁻¹ to 14 or 15 g·kg⁻¹·day⁻¹ all five rats developed ethanol overdose.

Fig. 8. Data from a rat fed ethanol, ephedrine, and caffeine (group 1) (A–F). A: daily UAL. B: liver showing acute centrilobular ischemic necrosis, H & E stain; magnification ×30. C: liver showing centrilobular bridging fibrosis, H & E stain; magnification ×30. D: liver showing centrilobular bridging fibrosis, reticulin stain, magnification ×6. E: liver showing centrilobular bridging fibrosis, reticulin stain; magnification ×30. F: liver showing a different focus, centrilobular bridging fibrosis, reticulin stain; magnification ×30.
Note that all the rats manifested the UAL cycle when given the 13 g·kg⁻¹·day⁻¹ ethanol dose. Cycles were not synchronized so that peaks and troughs occurred on different days in different rats. However, when the dose of ethanol was increased to 14 g·kg⁻¹·day⁻¹, or in the case of one rat 15 g·kg⁻¹·day⁻¹, the rats developed ethanol overdose as defined by death from high ethanol levels. When the dose of ethanol was increased from 13 g·kg⁻¹·day⁻¹, the UALs did not begin to rise until 15 g·kg⁻¹·day⁻¹ was given (Fig. 10). Ethanol overdose did not develop until a dose of 16–17 g·kg⁻¹·day⁻¹ of ethanol was given (16.4 ± 0.245) (Fig. 10). The difference between the lethal dose of ethanol for groups 1 and 3 was \( P < 0.001 \). Thus the ephedrine and caffeine treatment blocked the UAL cycle and increased the elimination rate of ethanol so that the rats could survive an otherwise lethal dose of ethanol. An example of the UALs of a rat fed ethanol (group 3) and a pair-fed rat fed ethanol, ephedrine, and caffeine is shown in Fig. 11. This is shown so that the individual pair-fed rats can be compared with each other.

**DISCUSSION**

As anticipated, ephedrine and caffeine feeding with ethanol prevented the UAL cycle and increased the dose of ethanol, which the rats could tolerate. The proposed explanation is that the increased metabolism caused by the elevated blood catecholamine levels prevented the cyclic changes in the metabolic rate caused by ethanol alone. This was similar to that seen when a high dose of thyroxine was fed with ethanol (7). That is, the catecholamine-induced increase in metabolic rate accelerated the rate of ethanol elimination, which prevented the urinary ethanol from reaching high levels. When high levels were reached by increasing the dose of ethanol, the hypothalamic-pituitary-thyroid mechanism of increasing the metabolic rate to increase the ethanol elimination rate was desensitized by the already increased metabolic rate. Thus the cycle was prevented from occurring.

Similar also to thyroxine feeding with ethanol, centrilobular ischemic necrosis of the liver was caused by ethanol plus ephedrine and caffeine feeding. This would suggest that the increased metabolic rate induced by feeding thyroxine or ephedrine plus caffeine increased the hypoxia observed in the liver at high blood ethanol levels as previously observed in the UAL cycle when the intragastric ethanol feeding model was used (1). The hypothesis that the ischemic necrosis observed was due to hypoxia is on the basis of I) the

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**Fig. 9.** The UAL levels of all 5 rats fed ethanol (group 3) are plotted. Note that all the rats manifested the UAL cycle when given the 13 g·kg⁻¹·day⁻¹ ethanol dose. Cycles were not synchronized so that peaks and troughs occurred on different days in different rats. However, when the dose of ethanol was increased to 14 g·kg⁻¹·day⁻¹, or in the case of one rat 15 g·kg⁻¹·day⁻¹, the rats developed ethanol overdose as defined by death from high ethanol levels.

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**Fig. 10.** The UAL levels of all 5 rats fed ethanol, ephedrine, and caffeine (group 1) are plotted. Note that the UAL cycle was completely prevented by this treatment. When the dose of ethanol was increased from 13 g·kg⁻¹·day⁻¹ to 14 and then 15 g·kg⁻¹·day⁻¹, the rats survived. Not until the ethanol dose of 16 g·kg⁻¹·day⁻¹ was given did the rats develop ethanol overdose. One rat required a dose of 17 g·kg⁻¹·day⁻¹ before eventually developing ethanol overdose.

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**Fig. 11.** The UAL levels of one rat fed ethanol and its pair-fed rat fed ethanol, ephedrine, and caffeine are plotted to better compare the data of individual rats. Note that the rat fed ethanol (group 3) showed the UAL cycle at a dose of ethanol at 13 g/day but overdosed when given 14 g·kg⁻¹·day⁻¹, whereas, the rat fed ethanol, ephedrine, and caffeine did not develop the UAL cycle and required a dose of 17 g·kg⁻¹·day⁻¹ ethanol before overdosing.
fact that hypoxia of the liver has been documented at high blood ethanol levels in this intragastric feeding rate model; and 2) the liver morphology of the central necrosis resembles that seen in ischemic necrosis observed in shock.

This observation may help explain the pathogenesis of liver toxicity observed in patients taking ephedrine and caffeine as a treatment for obesity (5). This phenomenon may put patients at risk who drink alcohol and take adrenergic drugs for obesity.

Experimentally, an acute dose of ethanol causes an elevation of both epinephrine and norepinephrine in mice (6). There is, again, an increase in plasma catecholamines in mice during ethanol withdrawal from chronic ethanol feeding (6). Thus in binge drinking the liver may be vulnerable to damage from hypoxia due to elevated endogenous plasma catecholamines during acute ethanol ingestion when high blood ethanol levels are achieved. This hypothesis is testable. In the present report, blood catecholamines were increased more with ethanol alone (group 3) compared with ephedrine plus caffeine fed alone (group 2). The combination of ethanol and ephedrine plus caffeine feeding (group 1) increased blood catecholamine levels indicating a synergistic effect. This was associated with increased liver pathology due to an enhanced degree of liver hypoxia that resulted.

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DISCLOSURES

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REFERENCES